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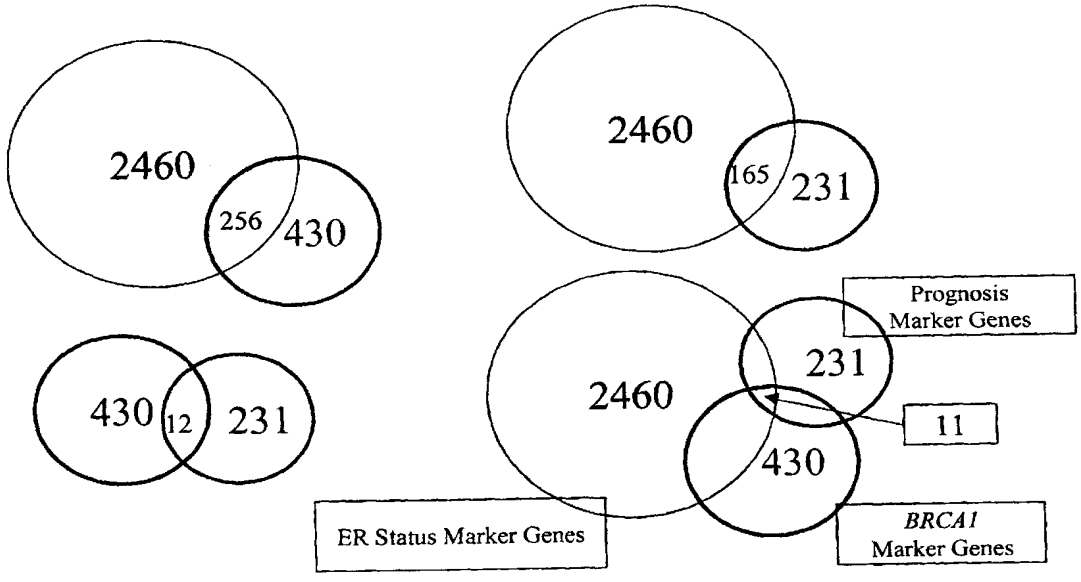

(57) Abstract: The present invention relates to genetic markers whose expression is correlated with breast cancer. Specifically, the invention provides sets of markers whose expression patterns can be used to differentiate clinical conditions associated with breast cancer, such as the presence or absence of the estrogen receptor *ESR1*, and *BRCA1* and sporadic tumors, and to provide information on the likelihood of tumor distant metastases within five years of initial diagnosis. The invention relates to methods of using these markers to distinguish these conditions. The invention also relates to kits containing ready-to-use microarrays and computer software for data analysis using the statistical methods disclosed herein.

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DIAGNOSIS AND PROGNOSIS OF BREAST CANCER PATIENTS

This application claims benefit of United States Provisional Application No. 60/298,918, filed June 18, 2001, and United States Provisional Application No. 60/380,710, filed on May 14, 2002, each of which is incorporated by reference herein in its entirety.

This application includes a Sequence Listing submitted on compact disc, recorded on two compact discs, including one duplicate, containing Filename 9301175228.txt, of size 6,755,971 bytes, created June 13, 2002. The sequence listing on the compact discs is incorporated by reference herein in its entirety.

1. FIELD OF THE INVENTION

The present invention relates to the identification of marker genes useful in the diagnosis and prognosis of breast cancer. More particularly, the invention relates to the identification of a set of marker genes associated with breast cancer, a set of marker genes differentially expressed in estrogen receptor (+) versus estrogen receptor (-) tumors, a set of marker genes differentially expressed in *BRCA1* versus sporadic tumors, and a set of marker genes differentially expressed in sporadic tumors from patients with good clinical prognosis (*i.e.*, metastasis- or disease-free >5 years) versus patients with poor clinical prognosis (*i.e.*, metastasis- or disease-free <5 years). For each of the marker sets above, the invention further relates to methods of distinguishing the breast cancer-related conditions. The invention further provides methods for determining the course of treatment of a patient with breast cancer.

2. BACKGROUND OF THE INVENTION

The increased number of cancer cases reported in the United States, and, indeed, around the world, is a major concern. Currently there are only a handful of treatments available for specific types of cancer, and these provide no guarantee of success. In order to be most effective, these treatments require not only an early detection of the malignancy, but a reliable assessment of the severity of the malignancy.

The incidence of breast cancer, a leading cause of death in women, has been gradually increasing in the United States over the last thirty years. Its cumulative risk is relatively high; 1 in 8 women are expected to develop some type of breast cancer by age 85

in the United States. In fact, breast cancer is the most common cancer in women and the second most common cause of cancer death in the United States. In 1997, it was estimated that 181,000 new cases were reported in the U.S., and that 44,000 people would die of breast cancer (Parker *et al., CA Cancer J. Clin.* 47:5-27 (1997); Chu *et al., J. Nat. Cancer Inst.* 88:1571-1579 (1996)). While mechanism of tumorigenesis for most breast carcinomas is largely unknown, there are genetic factors that can predispose some women to developing breast cancer (Miki *et al., Science*, 266:66-71(1994)). The discovery and characterization of *BRCA1* and *BRCA2* has recently expanded our knowledge of genetic factors which can contribute to familial breast cancer. Germ-line mutations within these two loci are associated with a 50 to 85% lifetime risk of breast and/or ovarian cancer (Casey, *Curr. Opin. Oncol.* 9:88-93 (1997); Marcus *et al., Cancer* 77:697-709 (1996)). Only about 5% to 10% of breast cancers are associated with breast cancer susceptibility genes, *BRCA1* and *BRCA2*. The cumulative lifetime risk of breast cancer for women who carry the mutant *BRCA1* is predicted to be approximately 92%, while the cumulative lifetime risk for the non-carrier majority is estimated to be approximately 10%. *BRCA1* is a tumor suppressor gene that is involved in DNA repair anc cell cycle control, which are both important for the maintenance of genomic stability. More than 90% of all mutations reported so far result in a premature truncation of the protein product with abnormal or abolished function. The histology of breast cancer in *BRCA1* mutation carriers differs from that in sporadic cases, but mutation analysis is the only way to find the carrier. Like *BRCA1*, *BRCA2* is involved in the development of breast cancer, and like *BRCA1* plays a role in DNA repair. However, unlike *BRCA1*, it is not involved in ovarian cancer.

Other genes have been linked to breast cancer, for example c-erb-2 (*HER2*) and p53 (Beenken et al., Ann. Surg. 233(5):630-638 (2001). Overexpression of c-erb-2 (*HER2*) and p53 have been correlated with poor prognosis (Rudolph et al., Hum. Pathol. 32(3):311-319 (2001), as has been aberrant expression products of *mdm2* (Lukas et al., Cancer Res. 61(7):3212-3219 (2001) and cyclin1 and p27 (Porter & Roberts, International Publication WO98/33450, published August 6, 1998). However, no other clinically useful markers consistently associated with breast cancer have been identified.

Sporadic tumors, those not currently associated with a known germline mutation, constitute the majority of breast cancers. It is also likely that other, non-genetic factors also have a significant effect on the etiology of the disease. Regardless of the cancer's origin, breast cancer morbidity and mortality increases significantly if it is not detected early in its progression. Thus, considerable effort has focused on the early detection of cellular transformation and tumor formation in breast tissue.

A marker-based approach to tumor identification and characterization promises improved diagnostic and prognostic reliability. Typically, the diagnosis of breast cancer requires histopathological proof of the presence of the tumor. In addition to diagnosis, histopathological examinations also provide information about prognosis and selection of treatment regimens. Prognosis may also be established based upon clinical parameters such as tumor size, tumor grade, the age of the patient, and lymph node metastasis.

Diagnosis and/or prognosis may be determined to varying degrees of effectiveness by direct examination of the outside of the breast, or through mammography or other X-ray imaging methods (Jatoi, *Am. J. Surg.* 177:518-524 (1999)). The latter approach is not without considerable cost, however. Every time a mammogram is taken, the patient incurs a small risk of having a breast tumor induced by the ionizing properties of the radiation used during the test. In addition, the process is expensive and the subjective interpretations of a technician can lead to imprecision. For example, one study showed major clinical disagreements for about one-third of a set of mammograms that were interpreted individually by a surveyed group of radiologists. Moreover, many women find that undergoing a mammogram is a painful experience. Accordingly, the National Cancer Institute has not recommended mammograms for women under fifty years of age, since this group is not as likely to develop breast cancers as are older women. It is compelling to note, however, that while only about 22% of breast cancers occur in women under fifty, data suggests that breast cancer is more aggressive in pre-menopausal women.

In clinical practice, accurate diagnosis of various subtypes of breast cancer is important because treatment options, prognosis, and the likelihood of therapeutic response all vary broadly depending on the diagnosis. Accurate prognosis, or determination of distant metastasis-free survival could allow the oncologist to tailor the administration of adjuvant chemotherapy, with women having poorer prognoses being given the most aggressive treatment. Furthermore, accurate prediction of poor prognosis would greatly impact clinical trials for new breast cancer therapies, because potential study patients could then be stratified according to prognosis. Trials could then be limited to patients having poor prognosis, in turn making it easier to discern if an experimental therapy is efficacious.

To date, no set of satisfactory predictors for prognosis based on the clinical information alone has been identified. The detection of *BRCA1* or *BRCA2* mutations represents a step towards the design of therapies to better control and prevent the appearance of these tumors. However, there is no equivalent means for the diagnosis of

patients with sporadic tumors, the most common type of breast cancer tumor, nor is there a means of differentiating subtypes of breast cancer.

3. SUMMARY OF THE INVENTION

The invention provides gene marker sets that distinguish various types and subtypes of breast cancer, and methods of use therefor. In one embodiment, the invention provides a method for classifying a cell sample as ER(+) or ER(-) comprising detecting a difference in the expression of a first plurality of genes relative to a control, said first plurality of genes consisting of at least 5 of the genes corresponding to the markers listed in Table 1. In specific embodiments, said plurality of genes consists of at least 50, 100, 200, 500, 1000, up to 2,460 of the gene markers listed in Table 1. In another specific embodiment, said plurality of genes consists of each of the genes corresponding to the 2,460 markers listed in Table 2. In another specific embodiment, said plurality consists of the 550 markers listed in Table 2. In another specific embodiment, said control comprises nucleic acids derived from a pool of tumors from individual sporadic patients. In another specific embodiment, said detecting comprises the steps of: (a) generating an ER(+) template by hybridization of nucleic acids derived from a plurality of ER(+) patients within a plurality of sporadic patients against nucleic acids derived from a pool of tumors from individual sporadic patients; (b) generating an ER(-) template by hybridization of nucleic acids derived from a plurality of ER(-) patients within said plurality of sporadic patients against nucleic acids derived from said pool of tumors from individual sporadic patients within said plurality; (c) hybridizing nucleic acids derived from an individual sample against said pool; and (d) determining the similarity of marker gene expression in the individual sample to the ER(+) template and the ER(-) template, wherein if said expression is more similar to the ER(+) template, the sample is classified as ER(+), and if said expression is more similar to the ER(-) template, the sample is classified as ER(-).

The invention further provides the above methods, applied to the classification of samples as *BRCA1* or sporadic, and classifying patients as having good prognosis or poor prognosis. For the *BRCA1*/sporadic gene markers, the invention provides that the method may be used wherein the plurality of genes is at least 5, 20, 50, 100, 200 or 300 of the *BRCA1*/sporadic markers listed in Table 3. In a specific embodiment, the optimum 100 markers listed in Table 4 are used. For the prognostic markers, the invention provides that at least 5, 20, 50, 100, or 200 gene markers listed in Table 5 may be used. In a specific embodiment, the optimum 70 markers listed in Table 6 are used.

The invention further provides that markers may be combined. Thus, in one embodiment, at least 5 markers from Table 1 are used in conjunction with at least 5 markers from Table 3. In another embodiment, at least 5 markers from Table 5 are used in conjunction with at least 5 markers from Table 3. In another embodiment, at least 5 markers from Table 1 are used in conjunction with at least 5 markers from Table 5. In another embodiment, at least 5 markers from each of Tables 1, 3, and 5 are used simultaneously.

The invention further provides a method for classifying a sample as ER(+) or ER(-) by calculating the similarity between the expression of at least 5 of the markers listed in Table 1 in the sample to the expression of the same markers in an ER(-) nucleic acid pool and an ER(+) nucleic acid pool, comprising the steps of: (a) labeling nucleic acids derived from a sample, with a first fluorophore to obtain a first pool of fluorophore-labeled nucleic acids; (b) labeling with a second fluorophore a first pool of nucleic acids derived from two or more ER(+) samples, and a second pool of nucleic acids derived from two or more ER(-) samples; (c) contacting said first fluorophore-labeled nucleic acid and said first pool of second fluorophore-labeled nucleic acid with said first microarray under conditions such that hybridization can occur, and contacting said first fluorophore-labeled nucleic acid and said second pool of second fluorophore-labeled nucleic acid with said second microarray under conditions such that hybridization can occur, detecting at each of a plurality of discrete loci on the first microarray a first flourescent emission signal from said first fluorophore-labeled nucleic acid and a second fluorescent emission signal from said first pool of second fluorophore-labeled genetic matter that is bound to said first microarray under said conditions, and detecting at each of the marker loci on said second microarray said first fluorescent emission signal from said first fluorophore-labeled nucleic acid and a third fluorescent emission signal from said second pool of second fluorophore-labeled nucleic acid; (d) determining the similarity of the sample to the ER(-) and ER(+) pools by comparing said first fluorescence emission signals and said second fluorescence emission signals, and said first emission signals and said third fluorescence emission signals; and (e) classifying the sample as ER(+) where the first fluorescence emission signals are more similar to said second fluorescence emission signals than to said third fluorescent emission signals, and classifying the sample as ER(-) where the first fluorescence emission signals are more similar to said third fluorescence emission signals than to said second fluorescent emission signals, wherein said similarity is defined by a statistical method. The invention further provides that the other disclosed marker sets may be used in the above method to

distinguish *BRCA1* from sporadic tumors, and patients with poor prognosis from patients with good prognosis.

In a specific embodiment, said similarity is calculated by determining a first sum of the differences of expression levels for each marker between said first fluorophore-labeled nucleic acid and said first pool of second fluorophore-labeled nucleic acid, and a second sum of the differences of expression levels for each marker between said first fluorophore-labeled nucleic acid and said second pool of second fluorophore-labeled nucleic acid, wherein if said first sum is greater than said second sum, the sample is classified as ER(-), and if said second sum is greater than said first sum, the sample is classified as ER(+). In another specific embodiment, said similarity is calculated by computing a first classifier parameter P_1 between an ER(+) template and the expression of said markers in said sample, and a second classifier parameter P_2 between an ER(-) template and the expression of said markers in said sample, wherein said P_1 and P_2 are calculated according to the formula:

$$P_i = (\vec{z}_i \bullet \vec{y}) / (\|\vec{z}_i\| \cdot \|\vec{y}\|), \qquad \text{Equation (1)}$$

wherein $\vec{z}_1$ and $\vec{z}_2$ are ER(-) and ER(+) templates, respectively, and are calculated by averaging said second fluorescence emission signal for each of said markers in said first pool of second fluorophore-labeled nucleic acid and said third fluorescence emission signal for each of said markers in said second pool of second fluorophore-labeled nucleic acid, respectively, and wherein $\vec{y}$ is said first fluorescence emission signal of each of said markers in the sample to be classified as ER(+) or ER(-), wherein the expression of the markers in the sample is similar to ER(+) if $P_1 < P_2$, and similar to ER(-) if $P_1 > P_2$.

The invention further provides a method for identifying marker genes the expression of which is associated with a particular phenotype. In one embodiment, the invention provides a method for determining a set of marker genes whose expression is associated with a particular phenotype, comprising the steps of: (a) selecting the phenotype having two or more phenotype categories; (b) identifying a plurality of genes wherein the expression of said genes is correlated or anticorrelated with one of the phenotype categories, and wherein the correlation coefficient for each gene is calculated according to the equation

$$\rho = (\vec{c} \bullet \vec{r}) / (\|\vec{c}\| \cdot \|\vec{r}\|) \qquad \text{Equation (2)}$$

wherein $\vec{c}$ is a number representing said phenotype category and $\vec{r}$ is the logarithmic expression ratio across all the samples for each individual gene, wherein if the correlation

coefficient has an absolute value of a threshold value or greater, said expression of said gene is associated with the phenotype category, and wherein said plurality of genes is a set of marker genes whose expression is associated with a particular phenotype. The threshold depends upon the number of samples used; the threshold can be calculated as 3 X $1/\sqrt{n-3}$, where $1/\sqrt{n-3}$ is the distribution width and n = the number of samples. In a specific embodiment where n = 98, said threshold value is 0.3. In a specific embodiment, said set of marker genes is validated by: (a) using a statistical method to randomize the association between said marker genes and said phenotype category, thereby creating a control correlation coefficient for each marker gene; (b) repeating step (a) one hundred or more times to develop a frequency distribution of said control correlation coefficients for each marker gene; (c) determining the number of marker genes having a control correlation coefficient of a threshold value or above, thereby creating a control marker gene set; and (d) comparing the number of control marker genes so identified to the number of marker genes, wherein if the p value of the difference between the number of marker genes and the number of control genes is less than 0.01, said set of marker genes is validated. In another specific embodiment, said set of marker genes is optimized by the method comprising: (a) rank-ordering the genes by amplitude of correlation or by significance of the correlation coefficients, and (b) selecting an arbitrary number of marker genes from the top of the rank-ordered list. The threshold value depends upon the number of samples tested.

The invention further provides a method for assigning a person to one of a plurality of categories in a clinical trial, comprising determining for each said person the level of expression of at least five of the prognosis markers listed in Table 6, determining therefrom whether the person has an expression pattern that correlates with a good prognosis or a poor prognosis, and assigning said person to one category in a clinical trial if said person is determined to have a good prognosis, and a different category if that person is determined to have a poor prognosis. The invention further provides a method for assigning a person to one of a plurality of categories in a clinical trial, where each of said categories is associated with a different phenotype, comprising determining for each said person the level of expression of at least five markers from a set of markers, wherein said set of markers includes markers associated with each of said clinical categories, determining therefrom whether the person has an expression pattern that correlates with one of the clinical categories, an assigning said person to one of said categories if said person is determined to have a phenotype associated with that category.

The invention further provides a method of classifying a first cell or organism as having one of at least two different phenotypes, said at least two different

phenotypes comprising a first phenotype and a second phenotype, said method comprising: (a) comparing the level of expression of each of a plurality of genes in a first sample from the first cell or organism to the level of expression of each of said genes, respectively, in a pooled sample from a plurality of cells or organisms, said plurality of cells or organisms comprising different cells or organisms exhibiting said at least two different phenotypes, respectively, to produce a first compared value; (b) comparing said first compared value to a second compared value, wherein said second compared value is the product of a method comprising comparing the level of expression of each of said genes in a sample from a cell or organism characterized as having said first phenotype to the level of expression of each of said genes, respectively, in said pooled sample; (c) comparing said first compared value to a third compared value, wherein said third compared value is the product of a method comprising comparing the level of expression of each of said genes in a sample from a cell or organism characterized as having said second phenotype to the level of expression of each of said genes, respectively, in said pooled sample, (d) optionally carrying out one or more times a step of comparing said first compared value to one or more additional compared values, respectively, each additional compared value being the product of a method comprising comparing the level of expression of each of said genes in a sample from a cell or organism characterized as having a phenotype different from said first and second phenotypes but included among said at least two different phenotypes, to the level of expression of each of said genes, respectively, in said pooled sample; and (e) determining to which of said second, third and, if present, one or more additional compared values, said first compared value is most similar, wherein said first cell or organism is determined to have the phenotype of the cell or organism used to produce said compared value most similar to said first compared value.

In a specific embodiment of the above method, said compared values are each ratios of the levels of expression of each of said genes. In another specific embodiment, each of said levels of expression of each of said genes in said pooled sample are normalized prior to any of said comparing steps. In another specific embodiment, normalizing said levels of expression is carried out by dividing each of said levels of expression by the median or mean level of expression of each of said genes or dividing by the mean or median level of expression of one or more housekeeping genes in said pooled sample. In a more specific embodiment, said normalized levels of expression are subjected to a log transform and said comparing steps comprise subtracting said log transform from the log of said levels of expression of each of said genes in said sample from said cell or organism. In another specific embodiment, said at least two different phenotypes are

different stages of a disease or disorder. In another specific embodiment, said at least two different phenotypes are different prognoses of a disease or disorder. In yet another specific embodiment, said levels of expression of each of said genes, respectively, in said pooled sample or said levels of expression of each of said genes in a sample from said cell or organism characterized as having said first phenotype, said second phenotype, or said phenotype different from said first and second phenotypes, respectively, are stored on a computer.

The invention further provides microarrays comprising the disclosed marker sets. In one embodiment, the invention provides a microarray comprising at least 5 markers derived from any one of Tables 1-6, wherein at least 50% of the probes on the microarray are present in any one of Tables 1-6. In more specific embodiments, at least 60%, 70%, 80%, 90%, 95% or 98% of the probes on said microarray are present in any one of Tables 1-6.

In another embodiment, the invention provides a microarray for distinguishing ER(+) and ER(-) cell samples comprising a positionally-addressable array of polynucleotide probes bound to a support, said polynucleotide probes comprising a plurality of polynucleotide probes of different nucleotide sequences, each of said different nucleotide sequences comprising a sequence complementary and hybridizable to a plurality of genes, said plurality consisting of at least 5 of the genes corresponding to the markers listed in Table 1 or Table 2, wherein at least 50% of the probes on the microarray are present in any one of Table 1 or Table 2. In yet another embodiment, the invention provides a microarray for distinguishing *BRCA1*-type and sporadic tumor-type cell samples comprising a positionally-addressable array of polynucleotide probes bound to a support, said polynucleotide probes comprising a plurality of polynucleotide probes of different nucleotide sequences, each of said different nucleotide sequences comprising a sequence complementary and hybridizable to a plurality of genes, said plurality consisting of at least 5 of the genes corresponding to the markers listed in Table 3 or Table 4, wherein at least 50% of the probes on the microarray are present in any one of Table 3 or Table 4. In still another embodiment, the invention provides a microarray for distinguishing cell samples from patients having a good prognosis and cell samples from patients having a poor prognosis comprising a positionally-addressable array of polynucleotide probes bound to a support, said polynucleotide probes comprising a plurality of polynucleotide probes of different nucleotide sequences, each of said different nucleotide sequences comprising a sequence complementary and hybridizable to a plurality of genes, said plurality consisting of at least 5 of the genes corresponding to the markers listed in Table 5 or Table 6, wherein at least 50%

of the probes on the microarray are present in any one of Table 5 or Table 6. The invention further provides for microarrays comprising at least 5, 20, 50, 100, 200, 500, 100, 1,250, 1,500, 1,750, or 2,000 of the ER-status marker genes listed in Table 1, at least 5, 20, 50, 100, 200, or 300 of the *BRCA1* sporadic marker genes listed in Table 3, or at least 5, 20, 50, 100 or 200 of the prognostic marker genes listed in Table 5, in any combination, wherein at least 50%, 60%, 70%, 80%, 90%, 95% or 98% of the probes on said microarrays are present in Table 1, Table 3 and/or Table 5.

The invention further provides a kit for determining the ER-status of a sample, comprising at least two microarrays each comprising at least 5 of the markers listed in Table 1, and a computer system for determining the similarity of the level of nucleic acid derived from the markers listed in Table 1 in a sample to that in an ER(-) pool and an ER(+) pool, the computer system comprising a processor, and a memory encoding one or more programs coupled to the processor, wherein the one or more programs cause the processor to perform a method comprising computing the aggregate differences in expression of each marker between the sample and ER(-) pool and the aggregate differences in expression of each marker between the sample and ER(+) pool, or a method comprising determining the correlation of expression of the markers in the sample to the expression in the ER(-) and ER(+) pools, said correlation calculated according to Equation (4). The invention provides for kits able to distinguish *BRCA1* and sporadic tumors, and samples from patients with good prognosis from samples from patients with poor prognosis, by inclusion of the appropriate marker gene sets. The invention further provides a kit for determining whether a sample is derived from a patient having a good prognosis or a poor prognosis, comprising at least one microarray comprising probes to at least 5 of the genes corresponding to the markers listed in Table 5, and a computer readable medium having recorded thereon one or more programs for determining the similarity of the level of nucleic acid derived from the markers listed in Table 5 in a sample to that in a pool of samples derived from individuals having a good prognosis and a pool of samples derived from individuals having a good prognosis, wherein the one or more programs cause a computer to perform a method comprising computing the aggregate differences in expression of each marker between the sample and the good prognosis pool and the aggregate differences in expression of each marker between the sample and the poor prognosis pool, or a method comprising determining the correlation of expression of the markers in the sample to the expression in the good prognosis and poor prognosis pools, said correlation calculated according to Equation (3).

4. BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 is a Venn-type diagram showing the overlap between the marker sets disclosed herein, including the 2,460 ER markers, the 430 *BRCA1*/sporadic markers, and the 231 prognosis reporters.

FIG. 2 shows the experimental procedures for measuring differential changes in mRNA transcript abundance in breast cancer tumors used in this study. In each experiment, Cy5-labeled cRNA from one tumor X is hybridized on a 25k human microarray together with a Cy3-labeled cRNA pool made of cRNA samples from tumors 1, 2, ... N. The digital expression data were obtained by scanning and image processing. The error modeling allowed us to assign a p-value to each transcript ratio measurement.

FIG. 3 Two-dimensional clustering reveals two distinctive types of tumors. The clustering was based on the gene expression data of 98 breast cancer tumors over 4986 significant genes. Dark gray (red) presents up-regulation, light gray (green) represents down-regulation, black indicates no change in expression, and gray indicates that data is not available. 4986 genes were selected that showed a more than two fold change in expression ratios in more than five experiments. Selected clinical data for test results of BR CA1 mutations, estrogen receptor (ER), and proestrogen receptor (PR), tumor grade, lymphocytic infiltrate, and angioinvasion are shown at right. Black denotes negative and white denotes positive. The dominant pattern in the lower part consists of 36 patients, out of which 34 are ER-negative (total 39), and 16 are BR CA1-mutation carriers (total 18).

FIG. 4 A portion of unsupervised clustered results as shown in FIG. 3. *ESR1* (the estrogen receptor gene) is coregulated with a set of genes that are strongly co-regulated to form a dominant pattern.

FIG. 5A Histogram of correlation coefficients of significant genes between their expression ratios and estrogen-receptor (ER) status (*i.e.*, ER level). The histogram for experimental data is shown as a gray line. The results of one Monte-Carlo trial is shown in solid black. There are 2,460 genes whose expression data correlate with ER status at a level higher than 0.3 or anti-correlated with ER status at a level lower than -0.3.

FIG. 5B The distribution of the number of genes that satisfied the same selection criteria (amplitude of correlation above 0.3) from 10,000 Monte-Carlo runs. It is estimated that this set of 2,460 genes reports ER status at a confidence level of $p > 99.99\%$.

FIG. 6 Classification Type 1 and Type 2 error rates as a function of the number (out of 2,460) marker genes used in the classifier. The combined error rate is lowest when approximately 550 marker genes are used.

FIG. 7 Classification of 98 tumor samples as ER(+) or ER(-) based on expression levels of the 550 optimal marker genes. ER(+) samples (above white line) exhibit a clearly different expression pattern that ER(-) samples (below white line).

FIG. 8 Correlation between expression levels in samples from each patient and the average profile of the ER(-) group vs. correlation with the ER(+) group. Squares represent samples from clinically ER(-) patients; dots represent samples from clinically ER(+) patients.

FIG. 9A Histogram of correlation coefficients of gene expression ratio of each significant gene with the *BRCA1* mutation status is shown as a solid line. The dashed line indicates a frequency distribution obtained from one Monte-Carlo run. 430 genes exhibited an amplitude of correlation or anti-correlation greater than 0.35.

FIG. 9B Frequency distribution of the number of genes that exhibit an amplitude of correlation or anti-correlation greater than 0.35 for the 10,000 Monte-Carlo run control. Mean = 115. $p(n > 430) = 0.48\%$ and $p(>430/2) = 9.0\%$.

FIG. 10 Classification type 1 and type 2 error rates as a function of the number of discriminating genes used in the classifier (template). The combined error rate is lowest when approximately 100 discriminating marker genes are used.

FIG. 11A The classification of 38 tumors in the ER(-) group into two subgroups, *BRCA1* and sporadic, by using the optimal set of 100 discriminating marker genes. Patients above the white line are characterized by *BRCA1*-related patterns.

FIG. 11B Correlation between expression levels in samples from each ER(-) patient and the average profile of the *BRCA1* group vs. correlation with the sporadic group. Squares represent samples from patients with sporadic-type tumors; dots represent samples from patients carrying the *BRCA1* mutation.

FIG. 12A Histogram of correlation coefficients of gene expression ratio of each significant gene with the prognostic category (distant metastases group and no distant metastases group) is shown as a solid line. The distribution obtained from one Monte-Carlo run is shown as a dashed line. The amplitude of correlation or anti-correlation of 231 marker genes is greater than 0.3.

FIG. 12B Frequency distribution of the number of genes whose amplitude of correlation or anti-correlation was greater than 0.3 for 10,000 Monte-Carlo runs.

FIG. 13 The distant metastases group classification error rate for type 1 and type 2 as a function of the number of discriminating genes used in the classifier. The combined error rate is lowest when approximately 70 discriminating marker genes are used.

FIG. 14 Classification of 78 sporadic tumors into two prognostic groups, distant metastases (poor prognosis) and no distant metastases (good prognosis) using the optimal set of 70 discriminating marker genes. Patients above the white line are characterized by good prognosis. Patients below the white line are characterized by poor prognosis.

FIG. 15 Correlation between expression levels in samples from each patient and the average profile of the good prognosis group vs. correlation with the poor prognosis group. Squares represent samples from patients having a poor prognosis; dots represent samples from patients having a good prognosis. Red squares represent the 'reoccurred' patients and the blue dots represent the 'non-reoccurred'. A total of 13 out of 78 were mis-classified.

FIG. 16 The reoccurrence probability as a function of time since diagnosis. Group A and group B were predicted by using a leave-one-out method based on the optimal set of 70 discriminating marker genes. The 43 patients in group A consists of 37 patients from the no distant metastases group and 6 patients from the distant metastases group. The 35 patients in group B consists of 28 patients from the distant metastases group and 7 patients from the no distant metastases group.

FIG. 17 The distant metastases probability as a function of time since diagnosis for ER(+) (yes) or ER(-) (no) individuals.

FIG. 18 The distant metastases probability as a function of time since diagnosis for progesterone receptor (PR)(+) (yes) or PR(-) (no) individuals.

FIG. 19A, B The distant metastases probability as a function of time since diagnosis. Groups were defined by the tumor grades.

FIG. 20A Classification of 19 independent sporadic tumors into two prognostic groups, distant metastases and no distant metastases, using the 70 optimal marker genes. Patients above the white line have a good prognosis. Patients below the white line have a poor prognosis.

FIG. 20B Correlation between expression ratios of each patient and the average expression ratio of the good prognosis group is defined by the training set versus the correlation between expression ratios of each patient and the average expression ratio of the poor prognosis training set. Of nine patients in the good prognosis group, three are from the "distant metastases group"; of ten patients in the good prognosis group, one patient is from the "no distant metastases group". This error rate of 4 out of 19 is consistent with 13 out of 78 for the initial 78 patients.

FIG. 20C The reoccurrence probability as a function of time since diagnosis for two groups predicted based on expression of the optimal 70 marker genes.

FIG. 21A Sensitivity vs. 1-specificity for good prognosis classification.

FIG. 21B Sensitivity vs. 1-specificity for poor prognosis classification.

FIG. 21C Total error rate as a function of threshold on the modeled likelihood. Six clinical parameters (ER status, PR status, tumor grade, tumor size, patient age, and presence or absence of angioinvasion) were used to perform the clinical modeling.

FIG. 22 Comparison of the log(ratio) of individual samples using the "material sample pool" vs. mean subtracted log(intensity) using the "mathematical sample pool" for 70 reporter genes in the 78 sporadic tumor samples. The "material sample pool" was constructed from the 78 sporadic tumor samples.

FIG. 23A Results of the "leave one out" cross validation based on single channel data. Samples are grouped according to each sample's coefficient of correlation to the average "good prognosis" profile and "poor prognosis" profile for the 70 genes examined. The white line separates samples from patients classified as having poor prognoses (below) and good prognoses (above).

FIG. 23B Scatter plot of coefficients of correlation to the average expression in "good prognosis" samples and "poor prognosis" samples. The false positive rate (*i.e.*, rate of incorrectly classifying a sample as being from a patient having a good prognosis as being one from a patient having a poor prognosis) was 10 out of 44, and the false negative rate is 6 out of 34.

FIG. 24A Single-channel hybridization data for samples ranked according to the coefficients of correlation with the good prognosis classifier. Samples classified as "good prognosis" lie above the white line, and those classified as "poor prognosis" lie below.

FIG. 24B Scatterplot of sample correlation coefficients, with three incorrectly classified samples lying to the right of the threshold correlation coefficient value. The threshold correlation value was set at 0.2727 to limit the false negatives to approximately 10% of the samples.

5. DETAILED DESCRIPTION OF THE INVENTION

5.1 INTRODUCTION

The invention relates to sets of genetic markers whose expression patterns correlate with important characteristics of breast cancer tumors. *i.e.*, estrogen receptor (ER) status, *BRCA1* status, and the likelihood of relapse (*i.e.*, distant metastasis or poor

prognosis). More specifically, the invention provides for sets of genetic markers that can distinguish the following three clinical conditions. First, the invention relates to sets of markers whose expression correlates with the ER status of a patient, and which can be used to distinguish ER(+) from ER(-) patients. ER status is a useful prognostic indicator, and an indicator of the likelihood that a patient will respond to certain therapies, such as tamoxifen. Also, among women who are ER positive the response rate (over 50%) to hormonal therapy is much higher than the response rate (less 10%) in patients whose ER status is negative. In patients with ER positive tumors the possibility of achieving a hormonal response is directly proportional to the level ER (P. Clabresi and P.S. Schein, MEDICAL ONCOLOGY (2ND ED.), McGraw-Hill, Inc., New York (1993)). Second, the invention further relates to sets of markers whose expression correlates with the presence of *BRCA1* mutations, and which can be used to distinguish *BRCA1*-type tumors from sporadic tumors. Third, the invention relates to genetic markers whose expression correlates with clinical prognosis, and which can be used to distinguish patients having good prognoses (*i.e.*, no distant metastases of a tumor within five years) from poor prognoses (*i.e.*, distant metastases of a tumor within five years). Methods are provided for use of these markers to distinguish between these patient groups, and to determine general courses of treatment. Microarrays comprising these markers are also provided, as well as methods of constructing such microarrays. Each markers correspond to a gene in the human genome, *i.e.*, such marker is identifiable as all or a portion of a gene. Finally, because each of the above markers correlates with a certain breast cancer-related conditions, the markers, or the proteins they encode, are likely to be targets for drugs against breast cancer.

5.2 DEFINITIONS

As used herein, "*BRCA1* tumor" means a tumor having cells containing a mutation of the *BRCA1* locus.

The "absolute amplitude" of correlation expressions means the distance, either positive or negative, from a zero value; *i.e.*, both correlation coefficients -0.35 and 0.35 have an absolute amplitude of 0.35.

"Status" means a state of gene expression of a set of genetic markers whose expression is strongly correlated with a particular phenotype. For example, "ER status" means a state of gene expression of a set of genetic markers whose expression is strongly correlated with that of *ESR1* (estrogen receptor gene), wherein the pattern of these genes' expression differs detectably between tumors expressing the receptor and tumors not expressing the receptor.

"Good prognosis" means that a patient is expected to have no distant metastases of a breast tumor within five years of initial diagnosis of breast cancer.

"Poor prognosis" means that a patient is expected to have distant metastases of a breast tumor within five years of initial diagnosis of breast cancer.

"Marker" means an entire gene, or an EST derived from that gene, the expression or level of which changes between certain conditions. Where the expression of the gene correlates with a certain condition, the gene is a marker for that condition.

"Marker-derived polynucleotides" means the RNA transcribed from a marker gene, any cDNA or cRNA produced therefrom, and any nucleic acid derived therefrom, such as synthetic nucleic acid having a sequence derived from the gene corresponding to the marker gene.

5.3 MARKERS USEFUL IN DIAGNOSIS AND PROGNOSIS OF BREAST CANCER

5.3.1 MARKER SETS

The invention provides a set of 4,986 genetic markers whose expression is correlated with the existence of breast cancer by clustering analysis. A subset of these markers identified as useful for diagnosis or prognosis is listed as SEQ ID NOS: 1-2,699. The invention also provides a method of using these markers to distinguish tumor types in diagnosis or prognosis.

In one embodiment, the invention provides a set of 2,460 genetic markers that can classify breast cancer patients by estrogen receptor (ER) status; *i.e.*, distinguish between ER(+) and ER(-) patients or tumors derived from these patients. ER status is an important indicator of the likelihood of a patient's response to some chemotherapies (*i.e.*, tamoxifen). These markers are listed in Table 1. The invention also provides subsets of at least 5, 10, 25, 50, 100, 200, 300, 400, 500, 750, 1,000, 1,250, 1,500, 1,750 or 2,000 genetic markers, drawn from the set of 2,460 markers, which also distinguish ER(+) and ER(-) patients or tumors. Preferably, the number of markers is 550. The invention further provides a set of 550 of the 2,460 markers that are optimal for distinguishing ER status (Table 2). The invention also provides a method of using these markers to distinguish between ER(+) and ER(-) patients or tumors derived therefrom.

In another embodiment, the invention provides a set of 430 genetic markers that can classify ER(-) breast cancer patients by *BRCA1* status; *i.e.*, distinguish between tumors containing a *BRCA1* mutation and sporadic tumors. These markers are listed in Table 3. The invention further provides subsets of at least 5, 10 20, 30, 40, 50, 75, 100, 150, 200, 250, 300 or 350 markers, drawn from the set of 430 markers, which also

distinguish between tumors containing a *BRCA1* mutation and sporadic tumors. Preferably, the number of markers is 100. A preferred set of 100 markers is provided in Table 4. The invention also provides a method of using these markers to distinguish between*BRCA1* and sporadic patients or tumors derived therefrom.

In another embodiment, the invention provides a set of 231 genetic markers that can distinguish between patients with a good breast cancer prognosis (no breast cancer tumor distant metastases within five years) and patients with a poor breast cancer prognosis (tumor distant metastases within five years). These markers are listed in Table 5. The invention also provides subsets of at least 5, 10, 20, 30, 40, 50, 75, 100, 150 or 200 markers, drawn from the set of 231, which also distinguish between patients with good and poor prognosis. A preferred set of 70 markers is provided in Table 6. In a specific embodiment, the set of markers consists of the twelve kinase-related markers and the seven cell division- or mitosis-related markers listed. The invention also provides a method of using the above markers to distinguish between patients with good or poor prognosis.

Table 1. 2,460 gene markers that distinguish ER(+) and ER(-) cell samples.

GenBank Accession Number	SEQ ID NO	GenBank Accession Number	SEQ ID NO
AA555029_RC	SEQ ID NO 1	NM_006984	SEQ ID NO 1344
AB000509	SEQ ID NO 2	NM_007005	SEQ ID NO 1345
AB001451	SEQ ID NO 3	NM_007006	SEQ ID NO 1346
AB002301	SEQ ID NO 4	NM_007019	SEQ ID NO 1347
AB002308	SEQ ID NO 5	NM_007027	SEQ ID NO 1348
AB002351	SEQ ID NO 6	NM_007044	SEQ ID NO 1350
AB002448	SEQ ID NO 7	NM_007050	SEQ ID NO 1351
AB006628	SEQ ID NO 9	NM_007057	SEQ ID NO 1352
AB006630	SEQ ID NO 10	NM_007069	SEQ ID NO 1353
AB006746	SEQ ID NO 11	NM_007074	SEQ ID NO 1355
AB007458	SEQ ID NO 12	NM_007088	SEQ ID NO 1356
AB007855	SEQ ID NO 13	NM_007111	SEQ ID NO 1357
AB007857	SEQ ID NO 14	NM_007146	SEQ ID NO 1358
AB007863	SEQ ID NO 15	NM_007173	SEQ ID NO 1359
AB007883	SEQ ID NO 16	NM_007177	SEQ ID NO 1360
AB007896	SEQ ID NO 17	NM_007196	SEQ ID NO 1361
AB007899	SEQ ID NO 18	NM_007203	SEQ ID NO 1362
AB007916	SEQ ID NO 19	NM_007214	SEQ ID NO 1363
AB007950	SEQ ID NO 20	NM_007217	SEQ ID NO 1364
AB011087	SEQ ID NO 21	NM_007231	SEQ ID NO 1365
AB011089	SEQ ID NO 22	NM_007268	SEQ ID NO 1367
AB011104	SEQ ID NO 23	NM_007274	SEQ ID NO 1368
AB011105	SEQ ID NO 24	NM_007275	SEQ ID NO 1369
AB011121	SEQ ID NO 25	NM_007281	SEQ ID NO 1370
AB011132	SEQ ID NO 26	NM_007309	SEQ ID NO 1371
AB011152	SEQ ID NO 27	NM_007315	SEQ ID NO 1372
AB011179	SEQ ID NO 28	NM_007334	SEQ ID NO 1373
AB014534	SEQ ID NO 29	NM_007358	SEQ ID NO 1374
AB014568	SEQ ID NO 30	NM_009585	SEQ ID NO 1375
AB018260	SEQ ID NO 31	NM_009587	SEQ ID NO 1376
AB018268	SEQ ID NO 32	NM_009588	SEQ ID NO 1377
AB018289	SEQ ID NO 33	NM_012062	SEQ ID NO 1378
AB018345	SEQ ID NO 35	NM_012067	SEQ ID NO 1379
AB020677	SEQ ID NO 36	NM_012101	SEQ ID NO 1380
AB020689	SEQ ID NO 37	NM_012105	SEQ ID NO 1381
AB020695	SEQ ID NO 38	NM_012108	SEQ ID NO 1382

GenBank Accession Number	SEQ ID NO	GenBank Accession Number	SEQ ID NO
AB020710	SEQ ID NO 39	NM_012110	SEQ ID NO 1383
AB023139	SEQ ID NO 40	NM_012124	SEQ ID NO 1384
AB023151	SEQ ID NO 41	NM_012142	SEQ ID NO 1386
AB023152	SEQ ID NO 42	NM_012155	SEQ ID NO 1388
AB023163	SEQ ID NO 43	NM_012175	SEQ ID NO 1389
AB023173	SEQ ID NO 44	NM_012177	SEQ ID NO 1390
AB023211	SEQ ID NO 45	NM_012205	SEQ ID NO 1391
AB024704	SEQ ID NO 46	NM_012219	SEQ ID NO 1393
AB028985	SEQ ID NO 47	NM_012242	SEQ ID NO 1394
AB028986	SEQ ID NO 48	NM_012250	SEQ ID NO 1395
AB028998	SEQ ID NO 49	NM_012261	SEQ ID NO 1397
AB029031	SEQ ID NO 51	NM_012286	SEQ ID NO 1398
AB032951	SEQ ID NO 52	NM_012319	SEQ ID NO 1400
AB032966	SEQ ID NO 53	NM_012332	SEQ ID NO 1401
AB032969	SEQ ID NO 54	NM_012336	SEQ ID NO 1402
AB032977	SEQ ID NO 56	NM_012339	SEQ ID NO 1404
AB033007	SEQ ID NO 58	NM_012341	SEQ ID NO 1405
AB033034	SEQ ID NO 59	NM_012391	SEQ ID NO 1406
AB033035	SEQ ID NO 60	NM_012394	SEQ ID NO 1407
AB033040	SEQ ID NO 61	NM_012413	SEQ ID NO 1408
AB033049	SEQ ID NO 63	NM_012421	SEQ ID NO 1409
AB033050	SEQ ID NO 64	NM_012425	SEQ ID NO 1410
AB033053	SEQ ID NO 65	NM_012427	SEQ ID NO 1411
AB033055	SEQ ID NO 66	NM_012429	SEQ ID NO 1413
AB033058	SEQ ID NO 67	NM_012446	SEQ ID NO 1414
AB033073	SEQ ID NO 68	NM_012463	SEQ ID NO 1415
AB033092	SEQ ID NO 69	NM_012474	SEQ ID NO 1416
AB033111	SEQ ID NO 70	NM_013230	SEQ ID NO 1417
AB036063	SEQ ID NO 71	NM_013233	SEQ ID NO 1418
AB037720	SEQ ID NO 72	NM_013238	SEQ ID NO 1419
AB037743	SEQ ID NO 74	NM_013239	SEQ ID NO 1420
AB037745	SEQ ID NO 75	NM_013242	SEQ ID NO 1421
AB037756	SEQ ID NO 76	NM_013257	SEQ ID NO 1423
AB037765	SEQ ID NO 77	NM_013261	SEQ ID NO 1424
AB037778	SEQ ID NO 78	NM_013262	SEQ ID NO 1425
AB037791	SEQ ID NO 79	NM_013277	SEQ ID NO 1426
AB037793	SEQ ID NO 80	NM_013296	SEQ ID NO 1427

GenBank Accession Number	SEQ ID NO	GenBank Accession Number	SEQ ID NO
AB037802	SEQ ID NO 81	NM_013301	SEQ ID NO 1428
AB037806	SEQ ID NO 82	NM_013324	SEQ ID NO 1429
AB037809	SEQ ID NO 83	NM_013327	SEQ ID NO 1430
AB037836	SEQ ID NO 84	NM_013336	SEQ ID NO 1431
AB037844	SEQ ID NO 85	NM_013339	SEQ ID NO 1432
AB037845	SEQ ID NO 86	NM_013363	SEQ ID NO 1433
AB037848	SEQ ID NO 87	NM_013378	SEQ ID NO 1435
AB037863	SEQ ID NO 88	NM_013384	SEQ ID NO 1436
AB037864	SEQ ID NO 89	NM_013385	SEQ ID NO 1437
AB040881	SEQ ID NO 90	NM_013406	SEQ ID NO 1438
AB040900	SEQ ID NO 91	NM_013437	SEQ ID NO 1439
AB040914	SEQ ID NO 92	NM_013451	SEQ ID NO 1440
AB040926	SEQ ID NO 93	NM_013943	SEQ ID NO 1441
AB040955	SEQ ID NO 94	NM_013994	SEQ ID NO 1442
AB040961	SEQ ID NO 95	NM_013995	SEQ ID NO 1443
AF000974	SEQ ID NO 97	NM_014026	SEQ ID NO 1444
AF005487	SEQ ID NO 98	NM_014029	SEQ ID NO 1445
AF007153	SEQ ID NO 99	NM_014036	SEQ ID NO 1446
AF007155	SEQ ID NO 100	NM_014062	SEQ ID NO 1447
AF015041	SEQ ID NO 101	NM_014074	SEQ ID NO 1448
AF016004	SEQ ID NO 102	NM_014096	SEQ ID NO 1450
AF016495	SEQ ID NO 103	NM_014109	SEQ ID NO 1451
AF020919	SEQ ID NO 104	NM_014112	SEQ ID NO 1452
AF026941	SEQ ID NO 105	NM_014147	SEQ ID NO 1453
AF035191	SEQ ID NO 106	NM_014149	SEQ ID NO 1454
AF035284	SEQ ID NO 107	NM_014164	SEQ ID NO 1455
AF035318	SEQ ID NO 108	NM_014172	SEQ ID NO 1456
AF038182	SEQ ID NO 109	NM_014175	SEQ ID NO 1457
AF038193	SEQ ID NO 110	NM_014181	SEQ ID NO 1458
AF042838	SEQ ID NO 111	NM_014184	SEQ ID NO 1459
AF044127	SEQ ID NO 112	NM_014211	SEQ ID NO 1460
AF045229	SEQ ID NO 113	NM_014214	SEQ ID NO 1461
AF047002	SEQ ID NO 114	NM_014216	SEQ ID NO 1462
AF047826	SEQ ID NO 115	NM_014241	SEQ ID NO 1463
AF049460	SEQ ID NO 116	NM_014246	SEQ ID NO 1465
AF052101	SEQ ID NO 117	NM_014268	SEQ ID NO 1466
AF052117	SEQ ID NO 118	NM_014272	SEQ ID NO 1467

GenBank Accession Number	SEQ ID NO	GenBank Accession Number	SEQ ID NO
AF052155	SEQ ID NO 119	NM_014274	SEQ ID NO 1468
AF052159	SEQ ID NO 120	NM_014289	SEQ ID NO 1469
AF052176	SEQ ID NO 122	NM_014298	SEQ ID NO 1470
AF052185	SEQ ID NO 123	NM_014302	SEQ ID NO 1471
AF055270	SEQ ID NO 126	NM_014315	SEQ ID NO 1473
AF058075	SEQ ID NO 127	NM_014316	SEQ ID NO 1474
AF061034	SEQ ID NO 128	NM_014317	SEQ ID NO 1475
AF063725	SEQ ID NO 129	NM_014320	SEQ ID NO 1476
AF063936	SEQ ID NO 130	NM_014321	SEQ ID NO 1477
AF065241	SEQ ID NO 131	NM_014325	SEQ ID NO 1478
AF067972	SEQ ID NO 132	NM_014335	SEQ ID NO 1479
AF070536	SEQ ID NO 133	NM_014363	SEQ ID NO 1480
AF070552	SEQ ID NO 134	NM_014364	SEQ ID NO 1481
AF070617	SEQ ID NO 135	NM_014365	SEQ ID NO 1482
AF073770	SEQ ID NO 138	NM_014373	SEQ ID NO 1483
AF076612	SEQ ID NO 139	NM_014382	SEQ ID NO 1484
AF079529	SEQ ID NO 140	NM_014395	SEQ ID NO 1485
AF090913	SEQ ID NO 142	NM_014398	SEQ ID NO 1486
AF095719	SEQ ID NO 143	NM_014399	SEQ ID NO 1487
AF098641	SEQ ID NO 144	NM_014402	SEQ ID NO 1488
AF099032	SEQ ID NO 145	NM_014428	SEQ ID NO 1489
AF100756	SEQ ID NO 146	NM_014448	SEQ ID NO 1490
AF101051	SEQ ID NO 147	NM_014449	SEQ ID NO 1491
AF103375	SEQ ID NO 148	NM_014450	SEQ ID NO 1492
AF103458	SEQ ID NO 149	NM_014452	SEQ ID NO 1493
AF103530	SEQ ID NO 150	NM_014453	SEQ ID NO 1494
AF103804	SEQ ID NO 151	NM_014456	SEQ ID NO 1495
AF111849	SEQ ID NO 152	NM_014479	SEQ ID NO 1497
AF112213	SEQ ID NO 153	NM_014501	SEQ ID NO 1498
AF113132	SEQ ID NO 154	NM_014552	SEQ ID NO 1500
AF116682	SEQ ID NO 156	NM_014553	SEQ ID NO 1501
AF118224	SEQ ID NO 157	NM_014570	SEQ ID NO 1502
AF118274	SEQ ID NO 158	NM_014575	SEQ ID NO 1503
AF119256	SEQ ID NO 159	NM_014585	SEQ ID NO 1504
AF119665	SEQ ID NO 160	NM_014595	SEQ ID NO 1505
AF121255	SEQ ID NO 161	NM_014624	SEQ ID NO 1507
AF131748	SEQ ID NO 162	NM_014633	SEQ ID NO 1508

GenBank Accession Number	SEQ ID NO	GenBank Accession Number	SEQ ID NO
AF131753	SEQ ID NO 163	NM_014640	SEQ ID NO 1509
AF131760	SEQ ID NO 164	NM_014642	SEQ ID NO 1510
AF131784	SEQ ID NO 165	NM_014643	SEQ ID NO 1511
AF131828	SEQ ID NO 166	NM_014656	SEQ ID NO 1512
AF135168	SEQ ID NO 167	NM_014668	SEQ ID NO 1513
AF141882	SEQ ID NO 168	NM_014669	SEQ ID NO 1514
AF148505	SEQ ID NO 169	NM_014673	SEQ ID NO 1515
AF149785	SEQ ID NO 170	NM_014675	SEQ ID NO 1516
AF151810	SEQ ID NO 171	NM_014679	SEQ ID NO 1517
AF152502	SEQ ID NO 172	NM_014680	SEQ ID NO 1518
AF155120	SEQ ID NO 174	NM_014696	SEQ ID NO 1519
AF159092	SEQ ID NO 175	NM_014700	SEQ ID NO 1520
AF161407	SEQ ID NO 176	NM_014715	SEQ ID NO 1521
AF161553	SEQ ID NO 177	NM_014721	SEQ ID NO 1522
AF164104	SEQ ID NO 178	NM_014737	SEQ ID NO 1524
AF167706	SEQ ID NO 179	NM_014738	SEQ ID NO 1525
AF175387	SEQ ID NO 180	NM_014747	SEQ ID NO 1526
AF176012	SEQ ID NO 181	NM_014750	SEQ ID NO 1527
AF186780	SEQ ID NO 182	NM_014754	SEQ ID NO 1528
AF217508	SEQ ID NO 184	NM_014767	SEQ ID NO 1529
AF220492	SEQ ID NO 185	NM_014770	SEQ ID NO 1530
AF224266	SEQ ID NO 186	NM_014773	SEQ ID NO 1531
AF230904	SEQ ID NO 187	NM_014776	SEQ ID NO 1532
AF234532	SEQ ID NO 188	NM_014782	SEQ ID NO 1533
AF257175	SEQ ID NO 189	NM_014785	SEQ ID NO 1534
AF257659	SEQ ID NO 190	NM_014791	SEQ ID NO 1535
AF272357	SEQ ID NO 191	NM_014808	SEQ ID NO 1536
AF279865	SEQ ID NO 192	NM_014811	SEQ ID NO 1537
AI497657_RC	SEQ ID NO 193	NM_014812	SEQ ID NO 1538
AJ012755	SEQ ID NO 194	NM_014838	SEQ ID NO 1540
AJ223353	SEQ ID NO 195	NM_014862	SEQ ID NO 1542
AJ224741	SEQ ID NO 196	NM_014865	SEQ ID NO 1543
AJ224864	SEQ ID NO 197	NM_014870	SEQ ID NO 1544
AJ225092	SEQ ID NO 198	NM_014875	SEQ ID NO 1545
AJ225093	SEQ ID NO 199	NM_014886	SEQ ID NO 1547
AJ249377	SEQ ID NO 200	NM_014889	SEQ ID NO 1548
AJ270996	SEQ ID NO 202	NM_014905	SEQ ID NO 1549

GenBank Accession Number	SEQ ID NO	GenBank Accession Number	SEQ ID NO
AJ272057	SEQ ID NO 203	NM_014935	SEQ ID NO 1550
AJ275978	SEQ ID NO 204	NM_014945	SEQ ID NO 1551
AJ276429	SEQ ID NO 205	NM_014965	SEQ ID NO 1552
AK000004	SEQ ID NO 206	NM_014967	SEQ ID NO 1553
AK000005	SEQ ID NO 207	NM_014968	SEQ ID NO 1554
AK000106	SEQ ID NO 208	NM_015032	SEQ ID NO 1555
AK000142	SEQ ID NO 209	NM_015239	SEQ ID NO 1556
AK000168	SEQ ID NO 210	NM_015383	SEQ ID NO 1557
AK000345	SEQ ID NO 212	NM_015392	SEQ ID NO 1558
AK000543	SEQ ID NO 213	NM_015416	SEQ ID NO 1559
AK000552	SEQ ID NO 214	NM_015417	SEQ ID NO 1560
AK000643	SEQ ID NO 216	NM_015420	SEQ ID NO 1561
AK000660	SEQ ID NO 217	NM_015434	SEQ ID NO 1562
AK000689	SEQ ID NO 218	NM_015474	SEQ ID NO 1563
AK000770	SEQ ID NO 220	NM_015507	SEQ ID NO 1565
AK000933	SEQ ID NO 221	NM_015513	SEQ ID NO 1566
AK001100	SEQ ID NO 223	NM_015515	SEQ ID NO 1567
AK001164	SEQ ID NO 224	NM_015523	SEQ ID NO 1568
AK001166	SEQ ID NO 225	NM_015524	SEQ ID NO 1569
AK001295	SEQ ID NO 226	NM_015599	SEQ ID NO 1571
AK001380	SEQ ID NO 227	NM_015623	SEQ ID NO 1572
AK001423	SEQ ID NO 228	NM_015640	SEQ ID NO 1573
AK001438	SEQ ID NO 229	NM_015641	SEQ ID NO 1574
AK001492	SEQ ID NO 230	NM_015678	SEQ ID NO 1575
AK001499	SEQ ID NO 231	NM_015721	SEQ ID NO 1576
AK001630	SEQ ID NO 232	NM_015892	SEQ ID NO 1578
AK001872	SEQ ID NO 234	NM_015895	SEQ ID NO 1579
AK001890	SEQ ID NO 235	NM_015907	SEQ ID NO 1580
AK002016	SEQ ID NO 236	NM_015925	SEQ ID NO 1581
AK002088	SEQ ID NO 237	NM_015937	SEQ ID NO 1582
AK002206	SEQ ID NO 240	NM_015954	SEQ ID NO 1583
AL035297	SEQ ID NO 241	NM_015955	SEQ ID NO 1584
AL049265	SEQ ID NO 242	NM_015961	SEQ ID NO 1585
AL049365	SEQ ID NO 244	NM_015984	SEQ ID NO 1587
AL049370	SEQ ID NO 245	NM_015986	SEQ ID NO 1588
AL049381	SEQ ID NO 246	NM_015987	SEQ ID NO 1589
AL049397	SEQ ID NO 247	NM_015991	SEQ ID NO 1590

GenBank Accession Number	SEQ ID NO	GenBank Accession Number	SEQ ID NO
AL049415	SEQ ID NO 248	NM_016002	SEQ ID NO 1592
AL049667	SEQ ID NO 249	NM_016028	SEQ ID NO 1594
AL049801	SEQ ID NO 250	NM_016029	SEQ ID NO 1595
AL049932	SEQ ID NO 251	NM_016047	SEQ ID NO 1596
AL049935	SEQ ID NO 252	NM_016048	SEQ ID NO 1597
AL049943	SEQ ID NO 253	NM_016050	SEQ ID NO 1598
AL049949	SEQ ID NO 254	NM_016056	SEQ ID NO 1599
AL049963	SEQ ID NO 255	NM_016058	SEQ ID NO 1600
AL049987	SEQ ID NO 256	NM_016066	SEQ ID NO 1601
AL050021	SEQ ID NO 257	NM_016072	SEQ ID NO 1602
AL050024	SEQ ID NO 258	NM_016073	SEQ ID NO 1603
AL050090	SEQ ID NO 259	NM_016108	SEQ ID NO 1605
AL050148	SEQ ID NO 260	NM_016109	SEQ ID NO 1606
AL050151	SEQ ID NO 261	NM_016121	SEQ ID NO 1607
AL050227	SEQ ID NO 262	NM_016126	SEQ ID NO 1608
AL050367	SEQ ID NO 263	NM_016127	SEQ ID NO 1609
AL050370	SEQ ID NO 264	NM_016135	SEQ ID NO 1610
AL050371	SEQ ID NO 265	NM_016142	SEQ ID NO 1612
AL050372	SEQ ID NO 266	NM_016153	SEQ ID NO 1613
AL050388	SEQ ID NO 267	NM_016171	SEQ ID NO 1614
AL079276	SEQ ID NO 268	NM_016175	SEQ ID NO 1615
AL079298	SEQ ID NO 269	NM_016184	SEQ ID NO 1616
AL080079	SEQ ID NO 271	NM_016185	SEQ ID NO 1617
AL080192	SEQ ID NO 273	NM_016187	SEQ ID NO 1618
AL080199	SEQ ID NO 274	NM_016199	SEQ ID NO 1619
AL080209	SEQ ID NO 275	NM_016210	SEQ ID NO 1620
AL080234	SEQ ID NO 277	NM_016217	SEQ ID NO 1621
AL080235	SEQ ID NO 278	NM_016228	SEQ ID NO 1623
AL096737	SEQ ID NO 279	NM_016229	SEQ ID NO 1624
AL110126	SEQ ID NO 280	NM_016235	SEQ ID NO 1625
AL110139	SEQ ID NO 281	NM_016240	SEQ ID NO 1626
AL110202	SEQ ID NO 283	NM_016243	SEQ ID NO 1627
AL110212	SEQ ID NO 284	NM_016250	SEQ ID NO 1628
AL110260	SEQ ID NO 285	NM_016267	SEQ ID NO 1629
AL117441	SEQ ID NO 286	NM_016271	SEQ ID NO 1630
AL117452	SEQ ID NO 287	NM_016299	SEQ ID NO 1631
AL117477	SEQ ID NO 288	NM_016306	SEQ ID NO 1632

GenBank Accession Number	SEQ ID NO	GenBank Accession Number	SEQ ID NO
AL117502	SEQ ID NO 289	NM_016308	SEQ ID NO 1634
AL117523	SEQ ID NO 290	NM_016321	SEQ ID NO 1635
AL117595	SEQ ID NO 291	NM_016337	SEQ ID NO 1636
AL117599	SEQ ID NO 292	NM_016352	SEQ ID NO 1637
AL117600	SEQ ID NO 293	NM_016359	SEQ ID NO 1638
AL117609	SEQ ID NO 294	NM_016401	SEQ ID NO 1641
AL117617	SEQ ID NO 295	NM_016403	SEQ ID NO 1642
AL117666	SEQ ID NO 296	NM_016411	SEQ ID NO 1643
AL122055	SEQ ID NO 297	NM_016423	SEQ ID NO 1644
AL133033	SEQ ID NO 298	NM_016463	SEQ ID NO 1647
AL133035	SEQ ID NO 299	NM_016475	SEQ ID NO 1649
AL133074	SEQ ID NO 301	NM_016477	SEQ ID NO 1650
AL133096	SEQ ID NO 302	NM_016491	SEQ ID NO 1651
AL133105	SEQ ID NO 303	NM_016495	SEQ ID NO 1652
AL133108	SEQ ID NO 304	NM_016542	SEQ ID NO 1653
AL133572	SEQ ID NO 305	NM_016548	SEQ ID NO 1654
AL133619	SEQ ID NO 307	NM_016569	SEQ ID NO 1655
AL133622	SEQ ID NO 308	NM_016577	SEQ ID NO 1656
AL133623	SEQ ID NO 309	NM_016582	SEQ ID NO 1657
AL133624	SEQ ID NO 310	NM_016593	SEQ ID NO 1658
AL133632	SEQ ID NO 311	NM_016603	SEQ ID NO 1659
AL133644	SEQ ID NO 312	NM_016612	SEQ ID NO 1660
AL133645	SEQ ID NO 313	NM_016619	SEQ ID NO 1661
AL133651	SEQ ID NO 314	NM_016623	SEQ ID NO 1663
AL137310	SEQ ID NO 316	NM_016625	SEQ ID NO 1664
AL137316	SEQ ID NO 317	NM_016629	SEQ ID NO 1665
AL137332	SEQ ID NO 318	NM_016640	SEQ ID NO 1666
AL137342	SEQ ID NO 319	NM_016645	SEQ ID NO 1667
AL137362	SEQ ID NO 321	NM_016650	SEQ ID NO 1668
AL137381	SEQ ID NO 322	NM_016657	SEQ ID NO 1669
AL137407	SEQ ID NO 323	NM_016733	SEQ ID NO 1670
AL137448	SEQ ID NO 324	NM_016815	SEQ ID NO 1671
AL137502	SEQ ID NO 326	NM_016817	SEQ ID NO 1672
AL137514	SEQ ID NO 327	NM_016818	SEQ ID NO 1673
AL137540	SEQ ID NO 328	NM_016839	SEQ ID NO 1675
AL137566	SEQ ID NO 330	NM_017414	SEQ ID NO 1676
AL137615	SEQ ID NO 331	NM_017422	SEQ ID NO 1677

GenBank Accession Number	SEQ ID NO	GenBank Accession Number	SEQ ID NO
AL137673	SEQ ID NO 335	NM_017423	SEQ ID NO 1678
AL137718	SEQ ID NO 336	NM_017447	SEQ ID NO 1679
AL137736	SEQ ID NO 337	NM_017518	SEQ ID NO 1680
AL137751	SEQ ID NO 338	NM_017522	SEQ ID NO 1681
AL137761	SEQ ID NO 339	NM_017540	SEQ ID NO 1682
AL157431	SEQ ID NO 340	NM_017555	SEQ ID NO 1683
AL157432	SEQ ID NO 341	NM_017572	SEQ ID NO 1684
AL157454	SEQ ID NO 342	NM_017585	SEQ ID NO 1685
AL157476	SEQ ID NO 343	NM_017586	SEQ ID NO 1686
AL157480	SEQ ID NO 344	NM_017596	SEQ ID NO 1687
AL157482	SEQ ID NO 345	NM_017606	SEQ ID NO 1688
AL157484	SEQ ID NO 346	NM_017617	SEQ ID NO 1689
AL157492	SEQ ID NO 347	NM_017633	SEQ ID NO 1690
AL157505	SEQ ID NO 348	NM_017634	SEQ ID NO 1691
AL157851	SEQ ID NO 349	NM_017646	SEQ ID NO 1692
AL160131	SEQ ID NO 350	NM_017660	SEQ ID NO 1693
AL161960	SEQ ID NO 351	NM_017680	SEQ ID NO 1694
AL162049	SEQ ID NO 352	NM_017691	SEQ ID NO 1695
AL355708	SEQ ID NO 353	NM_017698	SEQ ID NO 1696
D13643	SEQ ID NO 355	NM_017702	SEQ ID NO 1697
D14678	SEQ ID NO 356	NM_017731	SEQ ID NO 1699
D25328	SEQ ID NO 357	NM_017732	SEQ ID NO 1700
D26070	SEQ ID NO 358	NM_017733	SEQ ID NO 1701
D26488	SEQ ID NO 359	NM_017734	SEQ ID NO 1702
D31887	SEQ ID NO 360	NM_017746	SEQ ID NO 1703
D38521	SEQ ID NO 361	NM_017750	SEQ ID NO 1704
D38553	SEQ ID NO 362	NM_017761	SEQ ID NO 1705
D42043	SEQ ID NO 363	NM_017763	SEQ ID NO 1706
D42047	SEQ ID NO 364	NM_017770	SEQ ID NO 1707
D43950	SEQ ID NO 365	NM_017779	SEQ ID NO 1708
D50402	SEQ ID NO 366	NM_017780	SEQ ID NO 1709
D50914	SEQ ID NO 367	NM_017782	SEQ ID NO 1710
D55716	SEQ ID NO 368	NM_017786	SEQ ID NO 1711
D80001	SEQ ID NO 369	NM_017791	SEQ ID NO 1712
D80010	SEQ ID NO 370	NM_017805	SEQ ID NO 1713
D82345	SEQ ID NO 371	NM_017816	SEQ ID NO 1714
D83781	SEQ ID NO 372	NM_017821	SEQ ID NO 1715

GenBank Accession Number	SEQ ID NO	GenBank Accession Number	SEQ ID NO
D86964	SEQ ID NO 373	NM_017835	SEQ ID NO 1716
D86978	SEQ ID NO 374	NM_017843	SEQ ID NO 1717
D86985	SEQ ID NO 375	NM_017857	SEQ ID NO 1718
D87076	SEQ ID NO 376	NM_017901	SEQ ID NO 1719
D87453	SEQ ID NO 377	NM_017906	SEQ ID NO 1720
D87469	SEQ ID NO 378	NM_017918	SEQ ID NO 1721
D87682	SEQ ID NO 379	NM_017961	SEQ ID NO 1722
G26403	SEQ ID NO 380	NM_017996	SEQ ID NO 1723
J02639	SEQ ID NO 381	NM_018000	SEQ ID NO 1724
J04162	SEQ ID NO 382	NM_018004	SEQ ID NO 1725
K02403	SEQ ID NO 384	NM_018011	SEQ ID NO 1726
L05096	SEQ ID NO 385	NM_018014	SEQ ID NO 1727
L10333	SEQ ID NO 386	NM_018022	SEQ ID NO 1728
L11645	SEQ ID NO 387	NM_018031	SEQ ID NO 1729
L21934	SEQ ID NO 388	NM_018043	SEQ ID NO 1730
L22005	SEQ ID NO 389	NM_018048	SEQ ID NO 1731
L48692	SEQ ID NO 391	NM_018062	SEQ ID NO 1732
M12758	SEQ ID NO 392	NM_018069	SEQ ID NO 1733
M15178	SEQ ID NO 393	NM_018072	SEQ ID NO 1734
M21551	SEQ ID NO 394	NM_018077	SEQ ID NO 1735
M24895	SEQ ID NO 395	NM_018086	SEQ ID NO 1736
M26383	SEQ ID NO 396	NM_018087	SEQ ID NO 1737
M27749	SEQ ID NO 397	NM_018093	SEQ ID NO 1738
M28170	SEQ ID NO 398	NM_018098	SEQ ID NO 1739
M29873	SEQ ID NO 399	NM_018099	SEQ ID NO 1740
M29874	SEQ ID NO 400	NM_018101	SEQ ID NO 1741
M30448	SEQ ID NO 401	NM_018103	SEQ ID NO 1742
M30818	SEQ ID NO 402	NM_018109	SEQ ID NO 1744
M31932	SEQ ID NO 403	NM_018123	SEQ ID NO 1746
M37033	SEQ ID NO 404	NM_018131	SEQ ID NO 1747
M55914	SEQ ID NO 405	NM_018136	SEQ ID NO 1748
M63438	SEQ ID NO 406	NM_018138	SEQ ID NO 1749
M65254	SEQ ID NO 407	NM_018166	SEQ ID NO 1750
M68874	SEQ ID NO 408	NM_018171	SEQ ID NO 1751
M73547	SEQ ID NO 409	NM_018178	SEQ ID NO 1752
M77142	SEQ ID NO 410	NM_018181	SEQ ID NO 1753
M80899	SEQ ID NO 411	NM_018186	SEQ ID NO 1754

GenBank Accession Number	SEQ ID NO	GenBank Accession Number	SEQ ID NO
M83822	SEQ ID NO 412	NM_018188	SEQ ID NO 1756
M90657	SEQ ID NO 413	NM_018194	SEQ ID NO 1757
M93718	SEQ ID NO 414	NM_018204	SEQ ID NO 1758
M96577	SEQ ID NO 415	NM_018208	SEQ ID NO 1759
NM_000022	SEQ ID NO 417	NM_018212	SEQ ID NO 1760
NM_000044	SEQ ID NO 418	NM_018234	SEQ ID NO 1763
NM_000050	SEQ ID NO 419	NM_018255	SEQ ID NO 1764
NM_000057	SEQ ID NO 420	NM_018257	SEQ ID NO 1765
NM_000060	SEQ ID NO 421	NM_018265	SEQ ID NO 1766
NM_000064	SEQ ID NO 422	NM_018271	SEQ ID NO 1767
NM_000073	SEQ ID NO 424	NM_018290	SEQ ID NO 1768
NM_000077	SEQ ID NO 425	NM_018295	SEQ ID NO 1769
NM_000086	SEQ ID NO 426	NM_018304	SEQ ID NO 1770
NM_000087	SEQ ID NO 427	NM_018306	SEQ ID NO 1771
NM_000095	SEQ ID NO 429	NM_018326	SEQ ID NO 1772
NM_000096	SEQ ID NO 430	NM_018346	SEQ ID NO 1773
NM_000100	SEQ ID NO 431	NM_018366	SEQ ID NO 1775
NM_000101	SEQ ID NO 432	NM_018370	SEQ ID NO 1776
NM_000104	SEQ ID NO 433	NM_018373	SEQ ID NO 1777
NM_000109	SEQ ID NO 434	NM_018379	SEQ ID NO 1778
NM_000125	SEQ ID NO 435	NM_018384	SEQ ID NO 1779
NM_000127	SEQ ID NO 436	NM_018389	SEQ ID NO 1780
NM_000135	SEQ ID NO 437	NM_018410	SEQ ID NO 1783
NM_000137	SEQ ID NO 438	NM_018439	SEQ ID NO 1785
NM_000146	SEQ ID NO 439	NM_018454	SEQ ID NO 1786
NM_000149	SEQ ID NO 440	NM_018455	SEQ ID NO 1787
NM_000154	SEQ ID NO 441	NM_018465	SEQ ID NO 1788
NM_000161	SEQ ID NO 443	NM_018471	SEQ ID NO 1789
NM_000165	SEQ ID NO 444	NM_018478	SEQ ID NO 1790
NM_000168	SEQ ID NO 445	NM_018479	SEQ ID NO 1791
NM_000169	SEQ ID NO 446	NM_018529	SEQ ID NO 1793
NM_000175	SEQ ID NO 447	NM_018556	SEQ ID NO 1794
NM_000191	SEQ ID NO 448	NM_018569	SEQ ID NO 1795
NM_000201	SEQ ID NO 450	NM_018584	SEQ ID NO 1796
NM_000211	SEQ ID NO 451	NM_018653	SEQ ID NO 1797
NM_000213	SEQ ID NO 452	NM_018660	SEQ ID NO 1798
NM_000224	SEQ ID NO 453	NM_018683	SEQ ID NO 1799

GenBank Accession Number	SEQ ID NO	GenBank Accession Number	SEQ ID NO
NM_000239	SEQ ID NO 454	NM_018685	SEQ ID NO 1800
NM_000251	SEQ ID NO 455	NM_018686	SEQ ID NO 1801
NM_000268	SEQ ID NO 456	NM_018695	SEQ ID NO 1802
NM_000270	SEQ ID NO 458	NM_018728	SEQ ID NO 1803
NM_000271	SEQ ID NO 459	NM_018840	SEQ ID NO 1804
NM_000283	SEQ ID NO 460	NM_018842	SEQ ID NO 1805
NM_000284	SEQ ID NO 461	NM_018950	SEQ ID NO 1806
NM_000286	SEQ ID NO 462	NM_018988	SEQ ID NO 1807
NM_000291	SEQ ID NO 463	NM_019000	SEQ ID NO 1808
NM_000299	SEQ ID NO 464	NM_019013	SEQ ID NO 1809
NM_000300	SEQ ID NO 465	NM_019025	SEQ ID NO 1810
NM_000310	SEQ ID NO 466	NM_019027	SEQ ID NO 1811
NM_000311	SEQ ID NO 467	NM_019041	SEQ ID NO 1812
NM_000317	SEQ ID NO 468	NM_019044	SEQ ID NO 1813
NM_000320	SEQ ID NO 469	NM_019063	SEQ ID NO 1815
NM_000342	SEQ ID NO 470	NM_019084	SEQ ID NO 1816
NM_000346	SEQ ID NO 471	NM_019554	SEQ ID NO 1817
NM_000352	SEQ ID NO 472	NM_019845	SEQ ID NO 1818
NM_000355	SEQ ID NO 473	NM_019858	SEQ ID NO 1819
NM_000358	SEQ ID NO 474	NM_020130	SEQ ID NO 1820
NM_000359	SEQ ID NO 475	NM_020133	SEQ ID NO 1821
NM_000362	SEQ ID NO 476	NM_020143	SEQ ID NO 1822
NM_000365	SEQ ID NO 477	NM_020150	SEQ ID NO 1823
NM_000381	SEQ ID NO 478	NM_020163	SEQ ID NO 1824
NM_000397	SEQ ID NO 480	NM_020166	SEQ ID NO 1825
NM_000399	SEQ ID NO 481	NM_020169	SEQ ID NO 1826
NM_000414	SEQ ID NO 482	NM_020179	SEQ ID NO 1827
NM_000416	SEQ ID NO 483	NM_020184	SEQ ID NO 1828
NM_000422	SEQ ID NO 484	NM_020186	SEQ ID NO 1829
NM_000424	SEQ ID NO 485	NM_020188	SEQ ID NO 1830
NM_000433	SEQ ID NO 486	NM_020189	SEQ ID NO 1831
NM_000436	SEQ ID NO 487	NM_020197	SEQ ID NO 1832
NM_000450	SEQ ID NO 488	NM_020199	SEQ ID NO 1833
NM_000462	SEQ ID NO 489	NM_020215	SEQ ID NO 1834
NM_000495	SEQ ID NO 490	NM_020347	SEQ ID NO 1836
NM_000507	SEQ ID NO 491	NM_020365	SEQ ID NO 1837
NM_000526	SEQ ID NO 492	NM_020386	SEQ ID NO 1838

GenBank Accession Number	SEQ ID NO	GenBank Accession Number	SEQ ID NO
NM_000557	SEQ ID NO 493	NM_020445	SEQ ID NO 1839
NM_000560	SEQ ID NO 494	NM_020639	SEQ ID NO 1840
NM_000576	SEQ ID NO 495	NM_020659	SEQ ID NO 1841
NM_000579	SEQ ID NO 496	NM_020675	SEQ ID NO 1842
NM_000584	SEQ ID NO 497	NM_020686	SEQ ID NO 1843
NM_000591	SEQ ID NO 498	NM_020974	SEQ ID NO 1844
NM_000592	SEQ ID NO 499	NM_020978	SEQ ID NO 1845
NM_000593	SEQ ID NO 500	NM_020979	SEQ ID NO 1846
NM_000594	SEQ ID NO 501	NM_020980	SEQ ID NO 1847
NM_000597	SEQ ID NO 502	NM_021000	SEQ ID NO 1849
NM_000600	SEQ ID NO 504	NM_021004	SEQ ID NO 1850
NM_000607	SEQ ID NO 505	NM_021025	SEQ ID NO 1851
NM_000612	SEQ ID NO 506	NM_021063	SEQ ID NO 1852
NM_000627	SEQ ID NO 507	NM_021065	SEQ ID NO 1853
NM_000633	SEQ ID NO 508	NM_021077	SEQ ID NO 1854
NM_000636	SEQ ID NO 509	NM_021095	SEQ ID NO 1855
NM_000639	SEQ ID NO 510	NM_021101	SEQ ID NO 1856
NM_000647	SEQ ID NO 511	NM_021103	SEQ ID NO 1857
NM_000655	SEQ ID NO 512	NM_021128	SEQ ID NO 1858
NM_000662	SEQ ID NO 513	NM_021147	SEQ ID NO 1859
NM_000663	SEQ ID NO 514	NM_021151	SEQ ID NO 1860
NM_000666	SEQ ID NO 515	NM_021181	SEQ ID NO 1861
NM_000676	SEQ ID NO 516	NM_021190	SEQ ID NO 1862
NM_000685	SEQ ID NO 517	NM_021198	SEQ ID NO 1863
NM_000693	SEQ ID NO 518	NM_021200	SEQ ID NO 1864
NM_000699	SEQ ID NO 519	NM_021203	SEQ ID NO 1865
NM_000700	SEQ ID NO 520	NM_021238	SEQ ID NO 1866
NM_000712	SEQ ID NO 521	NM_021242	SEQ ID NO 1867
NM_000727	SEQ ID NO 522	S40706	SEQ ID NO 1869
NM_000732	SEQ ID NO 523	S53354	SEQ ID NO 1870
NM_000734	SEQ ID NO 524	S59184	SEQ ID NO 1871
NM_000767	SEQ ID NO 525	S62138	SEQ ID NO 1872
NM_000784	SEQ ID NO 526	U09848	SEQ ID NO 1873
NM_000802	SEQ ID NO 528	U10991	SEQ ID NO 1874
NM_000824	SEQ ID NO 529	U17077	SEQ ID NO 1875
NM_000849	SEQ ID NO 530	U18919	SEQ ID NO 1876
NM_000852	SEQ ID NO 531	U41387	SEQ ID NO 1877

GenBank Accession Number	SEQ ID NO	GenBank Accession Number	SEQ ID NO
NM_000874	SEQ ID NO 532	U45975	SEQ ID NO 1878
NM_000878	SEQ ID NO 533	U49835	SEQ ID NO 1879
NM_000884	SEQ ID NO 534	U56725	SEQ ID NO 1880
NM_000908	SEQ ID NO 537	U58033	SEQ ID NO 1881
NM_000909	SEQ ID NO 538	U61167	SEQ ID NO 1882
NM_000926	SEQ ID NO 539	U66042	SEQ ID NO 1883
NM_000930	SEQ ID NO 540	U68385	SEQ ID NO 1885
NM_000931	SEQ ID NO 541	U68494	SEQ ID NO 1886
NM_000947	SEQ ID NO 542	U74612	SEQ ID NO 1887
NM_000949	SEQ ID NO 543	U75968	SEQ ID NO 1888
NM_000950	SEQ ID NO 544	U79293	SEQ ID NO 1889
NM_000954	SEQ ID NO 545	U80736	SEQ ID NO 1890
NM_000964	SEQ ID NO 546	U82987	SEQ ID NO 1891
NM_001003	SEQ ID NO 549	U83115	SEQ ID NO 1892
NM_001016	SEQ ID NO 551	U89715	SEQ ID NO 1893
NM_001047	SEQ ID NO 553	U90916	SEQ ID NO 1894
NM_001066	SEQ ID NO 555	U92544	SEQ ID NO 1895
NM_001071	SEQ ID NO 556	U96131	SEQ ID NO 1896
NM_001078	SEQ ID NO 557	U96394	SEQ ID NO 1897
NM_001085	SEQ ID NO 558	W61000_RC	SEQ ID NO 1898
NM_001089	SEQ ID NO 559	X00437	SEQ ID NO 1899
NM_001109	SEQ ID NO 560	X00497	SEQ ID NO 1900
NM_001122	SEQ ID NO 561	X01394	SEQ ID NO 1901
NM_001124	SEQ ID NO 562	X03084	SEQ ID NO 1902
NM_001161	SEQ ID NO 563	X07834	SEQ ID NO 1905
NM_001165	SEQ ID NO 564	X14356	SEQ ID NO 1906
NM_001166	SEQ ID NO 565	X16302	SEQ ID NO 1907
NM_001168	SEQ ID NO 566	X52486	SEQ ID NO 1909
NM_001179	SEQ ID NO 567	X52882	SEQ ID NO 1910
NM_001185	SEQ ID NO 569	X56807	SEQ ID NO 1911
NM_001203	SEQ ID NO 570	X57809	SEQ ID NO 1912
NM_001207	SEQ ID NO 573	X57819	SEQ ID NO 1913
NM_001216	SEQ ID NO 574	X58529	SEQ ID NO 1914
NM_001218	SEQ ID NO 575	X59405	SEQ ID NO 1915
NM_001223	SEQ ID NO 576	X72475	SEQ ID NO 1918
NM_001225	SEQ ID NO 577	X73617	SEQ ID NO 1919
NM_001233	SEQ ID NO 578	X74794	SEQ ID NO 1920

GenBank Accession Number	SEQ ID NO	GenBank Accession Number	SEQ ID NO
NM_001236	SEQ ID NO 579	X75315	SEQ ID NO 1921
NM_001237	SEQ ID NO 580	X79782	SEQ ID NO 1922
NM_001251	SEQ ID NO 581	X82693	SEQ ID NO 1923
NM_001255	SEQ ID NO 582	X83301	SEQ ID NO 1924
NM_001262	SEQ ID NO 583	X93006	SEQ ID NO 1926
NM_001263	SEQ ID NO 584	X94232	SEQ ID NO 1927
NM_001267	SEQ ID NO 585	X98834	SEQ ID NO 1929
NM_001276	SEQ ID NO 587	X99142	SEQ ID NO 1930
NM_001280	SEQ ID NO 588	Y14737	SEQ ID NO 1932
NM_001282	SEQ ID NO 589	Z11887	SEQ ID NO 1933
NM_001295	SEQ ID NO 590	Z48633	SEQ ID NO 1935
NM_001305	SEQ ID NO 591	NM_004222	SEQ ID NO 1936
NM_001310	SEQ ID NO 592	NM_016405	SEQ ID NO 1937
NM_001312	SEQ ID NO 593	NM_017690	SEQ ID NO 1938
NM_001321	SEQ ID NO 594	Contig29_RC	SEQ ID NO 1939
NM_001327	SEQ ID NO 595	Contig237_RC	SEQ ID NO 1940
NM_001329	SEQ ID NO 596	Contig263_RC	SEQ ID NO 1941
NM_001333	SEQ ID NO 597	Contig292_RC	SEQ ID NO 1942
NM_001338	SEQ ID NO 598	Contig382_RC	SEQ ID NO 1944
NM_001360	SEQ ID NO 599	Contig399_RC	SEQ ID NO 1945
NM_001363	SEQ ID NO 600	Contig448_RC	SEQ ID NO 1946
NM_001381	SEQ ID NO 601	Contig569_RC	SEQ ID NO 1947
NM_001394	SEQ ID NO 602	Contig580_RC	SEQ ID NO 1948
NM_001395	SEQ ID NO 603	Contig678_RC	SEQ ID NO 1949
NM_001419	SEQ ID NO 604	Contig706_RC	SEQ ID NO 1950
NM_001424	SEQ ID NO 605	Contig718_RC	SEQ ID NO 1951
NM_001428	SEQ ID NO 606	Contig719_RC	SEQ ID NO 1952
NM_001436	SEQ ID NO 607	Contig742_RC	SEQ ID NO 1953
NM_001444	SEQ ID NO 608	Contig753_RC	SEQ ID NO 1954
NM_001446	SEQ ID NO 609	Contig758_RC	SEQ ID NO 1956
NM_001453	SEQ ID NO 611	Contig760_RC	SEQ ID NO 1957
NM_001456	SEQ ID NO 612	Contig842_RC	SEQ ID NO 1958
NM_001457	SEQ ID NO 613	Contig848_RC	SEQ ID NO 1959
NM_001463	SEQ ID NO 614	Contig924_RC	SEQ ID NO 1960
NM_001465	SEQ ID NO 615	Contig974_RC	SEQ ID NO 1961
NM_001481	SEQ ID NO 616	Contig1018_RC	SEQ ID NO 1962
NM_001493	SEQ ID NO 617	Contig1056_RC	SEQ ID NO 1963

GenBank Accession Number	SEQ ID NO	GenBank Accession Number	SEQ ID NO
NM_001494	SEQ ID NO 618	Contig1061_RC	SEQ ID NO 1964
NM_001500	SEQ ID NO 619	Contig1129_RC	SEQ ID NO 1965
NM_001504	SEQ ID NO 620	Contig1148	SEQ ID NO 1966
NM_001511	SEQ ID NO 621	Contig1239_RC	SEQ ID NO 1967
NM_001513	SEQ ID NO 622	Contig1277	SEQ ID NO 1968
NM_001527	SEQ ID NO 623	Contig1333_RC	SEQ ID NO 1969
NM_001529	SEQ ID NO 624	Contig1386_RC	SEQ ID NO 1970
NM_001530	SEQ ID NO 625	Contig1389_RC	SEQ ID NO 1971
NM_001540	SEQ ID NO 626	Contig1418_RC	SEQ ID NO 1972
NM_001550	SEQ ID NO 627	Contig1462_RC	SEQ ID NO 1973
NM_001551	SEQ ID NO 628	Contig1505_RC	SEQ ID NO 1974
NM_001552	SEQ ID NO 629	Contig1540_RC	SEQ ID NO 1975
NM_001554	SEQ ID NO 631	Contig1584_RC	SEQ ID NO 1976
NM_001558	SEQ ID NO 632	Contig1632_RC	SEQ ID NO 1977
NM_001560	SEQ ID NO 633	Contig1682_RC	SEQ ID NO 1978
NM_001565	SEQ ID NO 634	Contig1778_RC	SEQ ID NO 1979
NM_001569	SEQ ID NO 635	Contig1829	SEQ ID NO 1981
NM_001605	SEQ ID NO 636	Contig1838_RC	SEQ ID NO 1982
NM_001609	SEQ ID NO 637	Contig1938_RC	SEQ ID NO 1983
NM_001615	SEQ ID NO 638	Contig1970_RC	SEQ ID NO 1984
NM_001623	SEQ ID NO 639	Contig1998_RC	SEQ ID NO 1985
NM_001627	SEQ ID NO 640	Contig2099_RC	SEQ ID NO 1986
NM_001628	SEQ ID NO 641	Contig2143_RC	SEQ ID NO 1987
NM_001630	SEQ ID NO 642	Contig2237_RC	SEQ ID NO 1988
NM_001634	SEQ ID NO 643	Contig2429_RC	SEQ ID NO 1990
NM_001656	SEQ ID NO 644	Contig2504_RC	SEQ ID NO 1991
NM_001673	SEQ ID NO 645	Contig2512_RC	SEQ ID NO 1992
NM_001675	SEQ ID NO 647	Contig2575_RC	SEQ ID NO 1993
NM_001679	SEQ ID NO 648	Contig2578_RC	SEQ ID NO 1994
NM_001689	SEQ ID NO 649	Contig2639_RC	SEQ ID NO 1995
NM_001703	SEQ ID NO 650	Contig2647_RC	SEQ ID NO 1996
NM_001710	SEQ ID NO 651	Contig2657_RC	SEQ ID NO 1997
NM_001725	SEQ ID NO 652	Contig2728_RC	SEQ ID NO 1998
NM_001730	SEQ ID NO 653	Contig2745_RC	SEQ ID NO 1999
NM_001733	SEQ ID NO 654	Contig2811_RC	SEQ ID NO 2000
NM_001734	SEQ ID NO 655	Contig2873_RC	SEQ ID NO 2001
NM_001740	SEQ ID NO 656	Contig2883_RC	SEQ ID NO 2002

GenBank Accession Number	SEQ ID NO	GenBank Accession Number	SEQ ID NO
NM_001745	SEQ ID NO 657	Contig2915_RC	SEQ ID NO 2003
NM_001747	SEQ ID NO 658	Contig2928_RC	SEQ ID NO 2004
NM_001756	SEQ ID NO 659	Contig3024_RC	SEQ ID NO 2005
NM_001757	SEQ ID NO 660	Contig3094_RC	SEQ ID NO 2006
NM_001758	SEQ ID NO 661	Contig3164_RC	SEQ ID NO 2007
NM_001762	SEQ ID NO 662	Contig3495_RC	SEQ ID NO 2009
NM_001767	SEQ ID NO 663	Contig3607_RC	SEQ ID NO 2010
NM_001770	SEQ ID NO 664	Contig3659_RC	SEQ ID NO 2011
NM_001777	SEQ ID NO 665	Contig3677_RC	SEQ ID NO 2012
NM_001778	SEQ ID NO 666	Contig3682_RC	SEQ ID NO 2013
NM_001781	SEQ ID NO 667	Contig3734_RC	SEQ ID NO 2014
NM_001786	SEQ ID NO 668	Contig3834_RC	SEQ ID NO 2015
NM_001793	SEQ ID NO 669	Contig3876_RC	SEQ ID NO 2016
NM_001803	SEQ ID NO 671	Contig3902_RC	SEQ ID NO 2017
NM_001806	SEQ ID NO 672	Contig3940_RC	SEQ ID NO 2018
NM_001809	SEQ ID NO 673	Contig4380_RC	SEQ ID NO 2019
NM_001814	SEQ ID NO 674	Contig4388_RC	SEQ ID NO 2020
NM_001826	SEQ ID NO 675	Contig4467_RC	SEQ ID NO 2021
NM_001830	SEQ ID NO 677	Contig4949_RC	SEQ ID NO 2023
NM_001838	SEQ ID NO 678	Contig5348_RC	SEQ ID NO 2024
NM_001839	SEQ ID NO 679	Contig5403_RC	SEQ ID NO 2025
NM_001853	SEQ ID NO 681	Contig5716_RC	SEQ ID NO 2026
NM_001859	SEQ ID NO 682	Contig6118_RC	SEQ ID NO 2027
NM_001861	SEQ ID NO 683	Contig6164_RC	SEQ ID NO 2028
NM_001874	SEQ ID NO 685	Contig6181_RC	SEQ ID NO 2029
NM_001885	SEQ ID NO 686	Contig6514_RC	SEQ ID NO 2030
NM_001892	SEQ ID NO 688	Contig6612_RC	SEQ ID NO 2031
NM_001897	SEQ ID NO 689	Contig6881_RC	SEQ ID NO 2032
NM_001899	SEQ ID NO 690	Contig8165_RC	SEQ ID NO 2033
NM_001905	SEQ ID NO 691	Contig8221_RC	SEQ ID NO 2034
NM_001912	SEQ ID NO 692	Contig8347_RC	SEQ ID NO 2035
NM_001914	SEQ ID NO 693	Contig8364_RC	SEQ ID NO 2036
NM_001919	SEQ ID NO 694	Contig8888_RC	SEQ ID NO 2038
NM_001941	SEQ ID NO 695	Contig9259_RC	SEQ ID NO 2039
NM_001943	SEQ ID NO 696	Contig9541_RC	SEQ ID NO 2040
NM_001944	SEQ ID NO 697	Contig10268_RC	SEQ ID NO 2041
NM_001953	SEQ ID NO 699	Contig10363_RC	SEQ ID NO 2042

GenBank Accession Number	SEQ ID NO	GenBank Accession Number	SEQ ID NO
NM_001954	SEQ ID NO 700	Contig10437_RC	SEQ ID NO 2043
NM_001955	SEQ ID NO 701	Contig11086_RC	SEQ ID NO 2045
NM_001956	SEQ ID NO 702	Contig11275_RC	SEQ ID NO 2046
NM_001958	SEQ ID NO 703	Contig11648_RC	SEQ ID NO 2047
NM_001961	SEQ ID NO 705	Contig12216_RC	SEQ ID NO 2048
NM_001970	SEQ ID NO 706	Contig12369_RC	SEQ ID NO 2049
NM_001979	SEQ ID NO 707	Contig12814_RC	SEQ ID NO 2050
NM_001982	SEQ ID NO 708	Contig12951_RC	SEQ ID NO 2051
NM_002017	SEQ ID NO 710	Contig13480_RC	SEQ ID NO 2052
NM_002033	SEQ ID NO 713	Contig14284_RC	SEQ ID NO 2053
NM_002046	SEQ ID NO 714	Contig14390_RC	SEQ ID NO 2054
NM_002047	SEQ ID NO 715	Contig14780_RC	SEQ ID NO 2055
NM_002051	SEQ ID NO 716	Contig14954_RC	SEQ ID NO 2056
NM_002053	SEQ ID NO 717	Contig14981_RC	SEQ ID NO 2057
NM_002061	SEQ ID NO 718	Contig15692_RC	SEQ ID NO 2058
NM_002065	SEQ ID NO 719	Contig16192_RC	SEQ ID NO 2059
NM_002068	SEQ ID NO 720	Contig16759_RC	SEQ ID NO 2061
NM_002077	SEQ ID NO 722	Contig16786_RC	SEQ ID NO 2062
NM_002091	SEQ ID NO 723	Contig16905_RC	SEQ ID NO 2063
NM_002101	SEQ ID NO 724	Contig17103_RC	SEQ ID NO 2064
NM_002106	SEQ ID NO 725	Contig17105_RC	SEQ ID NO 2065
NM_002110	SEQ ID NO 726	Contig17248_RC	SEQ ID NO 2066
NM_002111	SEQ ID NO 727	Contig17345_RC	SEQ ID NO 2067
NM_002115	SEQ ID NO 728	Contig18502_RC	SEQ ID NO 2069
NM_002118	SEQ ID NO 729	Contig20156_RC	SEQ ID NO 2071
NM_002123	SEQ ID NO 730	Contig20302_RC	SEQ ID NO 2073
NM_002131	SEQ ID NO 731	Contig20600_RC	SEQ ID NO 2074
NM_002136	SEQ ID NO 732	Contig20617_RC	SEQ ID NO 2075
NM_002145	SEQ ID NO 733	Contig20629_RC	SEQ ID NO 2076
NM_002164	SEQ ID NO 734	Contig20651_RC	SEQ ID NO 2077
NM_002168	SEQ ID NO 735	Contig21130_RC	SEQ ID NO 2078
NM_002184	SEQ ID NO 736	Contig21185_RC	SEQ ID NO 2079
NM_002185	SEQ ID NO 737	Contig21421_RC	SEQ ID NO 2080
NM_002189	SEQ ID NO 738	Contig21787_RC	SEQ ID NO 2081
NM_002200	SEQ ID NO 739	Contig21812_RC	SEQ ID NO 2082
NM_002201	SEQ ID NO 740	Contig22418_RC	SEQ ID NO 2083
NM_002213	SEQ ID NO 741	Contig23085_RC	SEQ ID NO 2084

GenBank Accession Number	SEQ ID NO	GenBank Accession Number	SEQ ID NO
NM_002219	SEQ ID NO 742	Contig23454_RC	SEQ ID NO 2085
NM_002222	SEQ ID NO 743	Contig24138_RC	SEQ ID NO 2086
NM_002239	SEQ ID NO 744	Contig24252_RC	SEQ ID NO 2087
NM_002243	SEQ ID NO 745	Contig24655_RC	SEQ ID NO 2089
NM_002245	SEQ ID NO 746	Contig25055_RC	SEQ ID NO 2090
NM_002250	SEQ ID NO 747	Contig25290_RC	SEQ ID NO 2091
NM_002254	SEQ ID NO 748	Contig25343_RC	SEQ ID NO 2092
NM_002266	SEQ ID NO 749	Contig25362_RC	SEQ ID NO 2093
NM_002273	SEQ ID NO 750	Contig25617_RC	SEQ ID NO 2094
NM_002281	SEQ ID NO 751	Contig25659_RC	SEQ ID NO 2095
NM_002292	SEQ ID NO 752	Contig25722_RC	SEQ ID NO 2096
NM_002298	SEQ ID NO 753	Contig25809_RC	SEQ ID NO 2097
NM_002300	SEQ ID NO 754	Contig25991	SEQ ID NO 2098
NM_002308	SEQ ID NO 755	Contig26022_RC	SEQ ID NO 2099
NM_002314	SEQ ID NO 756	Contig26077_RC	SEQ ID NO 2100
NM_002337	SEQ ID NO 757	Contig26310_RC	SEQ ID NO 2101
NM_002341	SEQ ID NO 758	Contig26371_RC	SEQ ID NO 2102
NM_002342	SEQ ID NO 759	Contig26438_RC	SEQ ID NO 2103
NM_002346	SEQ ID NO 760	Contig26706_RC	SEQ ID NO 2104
NM_002349	SEQ ID NO 761	Contig27088_RC	SEQ ID NO 2105
NM_002350	SEQ ID NO 762	Contig27186_RC	SEQ ID NO 2106
NM_002356	SEQ ID NO 763	Contig27228_RC	SEQ ID NO 2107
NM_002358	SEQ ID NO 764	Contig27344_RC	SEQ ID NO 2109
NM_002370	SEQ ID NO 765	Contig27386_RC	SEQ ID NO 2110
NM_002395	SEQ ID NO 766	Contig27624_RC	SEQ ID NO 2111
NM_002416	SEQ ID NO 767	Contig27749_RC	SEQ ID NO 2112
NM_002421	SEQ ID NO 768	Contig27882_RC	SEQ ID NO 2113
NM_002426	SEQ ID NO 769	Contig27915_RC	SEQ ID NO 2114
NM_002435	SEQ ID NO 770	Contig28030_RC	SEQ ID NO 2115
NM_002438	SEQ ID NO 771	Contig28081_RC	SEQ ID NO 2116
NM_002444	SEQ ID NO 772	Contig28152_RC	SEQ ID NO 2117
NM_002449	SEQ ID NO 773	Contig28550_RC	SEQ ID NO 2119
NM_002450	SEQ ID NO 774	Contig28552_RC	SEQ ID NO 2120
NM_002456	SEQ ID NO 775	Contig28712_RC	SEQ ID NO 2121
NM_002466	SEQ ID NO 776	Contig28888_RC	SEQ ID NO 2122
NM_002482	SEQ ID NO 777	Contig28947_RC	SEQ ID NO 2123
NM_002497	SEQ ID NO 778	Contig29126_RC	SEQ ID NO 2124

GenBank Accession Number	SEQ ID NO	GenBank Accession Number	SEQ ID NO
NM_002510	SEQ ID NO 779	Contig29193_RC	SEQ ID NO 2125
NM_002515	SEQ ID NO 781	Contig29369_RC	SEQ ID NO 2126
NM_002524	SEQ ID NO 782	Contig29639_RC	SEQ ID NO 2127
NM_002539	SEQ ID NO 783	Contig30047_RC	SEQ ID NO 2129
NM_002555	SEQ ID NO 785	Contig30154_RC	SEQ ID NO 2131
NM_002570	SEQ ID NO 787	Contig30209_RC	SEQ ID NO 2132
NM_002579	SEQ ID NO 788	Contig30213_RC	SEQ ID NO 2133
NM_002587	SEQ ID NO 789	Contig30230_RC	SEQ ID NO 2134
NM_002590	SEQ ID NO 790	Contig30267_RC	SEQ ID NO 2135
NM_002600	SEQ ID NO 791	Contig30390_RC	SEQ ID NO 2136
NM_002614	SEQ ID NO 792	Contig30480_RC	SEQ ID NO 2137
NM_002618	SEQ ID NO 794	Contig30609_RC	SEQ ID NO 2138
NM_002626	SEQ ID NO 795	Contig30934_RC	SEQ ID NO 2139
NM_002633	SEQ ID NO 796	Contig31150_RC	SEQ ID NO 2140
NM_002639	SEQ ID NO 797	Contig31186_RC	SEQ ID NO 2141
NM_002648	SEQ ID NO 798	Contig31251_RC	SEQ ID NO 2142
NM_002659	SEQ ID NO 799	Contig31288_RC	SEQ ID NO 2143
NM_002661	SEQ ID NO 800	Contig31291_RC	SEQ ID NO 2144
NM_002662	SEQ ID NO 801	Contig31295_RC	SEQ ID NO 2145
NM_002664	SEQ ID NO 802	Contig31424_RC	SEQ ID NO 2146
NM_002689	SEQ ID NO 804	Contig31449_RC	SEQ ID NO 2147
NM_002690	SEQ ID NO 805	Contig31596_RC	SEQ ID NO 2148
NM_002709	SEQ ID NO 806	Contig31864_RC	SEQ ID NO 2149
NM_002727	SEQ ID NO 807	Contig31928_RC	SEQ ID NO 2150
NM_002729	SEQ ID NO 808	Contig31966_RC	SEQ ID NO 2151
NM_002734	SEQ ID NO 809	Contig31986_RC	SEQ ID NO 2152
NM_002736	SEQ ID NO 810	Contig32084_RC	SEQ ID NO 2153
NM_002740	SEQ ID NO 811	Contig32105_RC	SEQ ID NO 2154
NM_002748	SEQ ID NO 813	Contig32185_RC	SEQ ID NO 2156
NM_002774	SEQ ID NO 814	Contig32242_RC	SEQ ID NO 2157
NM_002775	SEQ ID NO 815	Contig32322_RC	SEQ ID NO 2158
NM_002776	SEQ ID NO 816	Contig32336_RC	SEQ ID NO 2159
NM_002789	SEQ ID NO 817	Contig32558_RC	SEQ ID NO 2160
NM_002794	SEQ ID NO 818	Contig32798_RC	SEQ ID NO 2161
NM_002796	SEQ ID NO 819	Contig33005_RC	SEQ ID NO 2162
NM_002800	SEQ ID NO 820	Contig33230_RC	SEQ ID NO 2163
NM_002801	SEQ ID NO 821	Contig33260_RC	SEQ ID NO 2164

GenBank Accession Number	SEQ ID NO	GenBank Accession Number	SEQ ID NO
NM_002808	SEQ ID NO 822	Contig33654_RC	SEQ ID NO 2166
NM_002821	SEQ ID NO 824	Contig33741_RC	SEQ ID NO 2167
NM_002826	SEQ ID NO 825	Contig33771_RC	SEQ ID NO 2168
NM_002827	SEQ ID NO 826	Contig33814_RC	SEQ ID NO 2169
NM_002838	SEQ ID NO 827	Contig33815_RC	SEQ ID NO 2170
NM_002852	SEQ ID NO 828	Contig33833	SEQ ID NO 2171
NM_002854	SEQ ID NO 829	Contig33998_RC	SEQ ID NO 2172
NM_002856	SEQ ID NO 830	Contig34079	SEQ ID NO 2173
NM_002857	SEQ ID NO 831	Contig34080_RC	SEQ ID NO 2174
NM_002858	SEQ ID NO 832	Contig34222_RC	SEQ ID NO 2175
NM_002888	SEQ ID NO 833	Contig34233_RC	SEQ ID NO 2176
NM_002890	SEQ ID NO 834	Contig34303_RC	SEQ ID NO 2177
NM_002901	SEQ ID NO 836	Contig34393_RC	SEQ ID NO 2178
NM_002906	SEQ ID NO 837	Contig34477_RC	SEQ ID NO 2179
NM_002916	SEQ ID NO 838	Contig34766_RC	SEQ ID NO 2181
NM_002923	SEQ ID NO 839	Contig34952	SEQ ID NO 2182
NM_002933	SEQ ID NO 840	Contig34989_RC	SEQ ID NO 2183
NM_002936	SEQ ID NO 841	Contig35030_RC	SEQ ID NO 2184
NM_002937	SEQ ID NO 842	Contig35251_RC	SEQ ID NO 2185
NM_002950	SEQ ID NO 843	Contig35629_RC	SEQ ID NO 2186
NM_002961	SEQ ID NO 844	Contig35635_RC	SEQ ID NO 2187
NM_002964	SEQ ID NO 845	Contig35763_RC	SEQ ID NO 2188
NM_002965	SEQ ID NO 846	Contig35814_RC	SEQ ID NO 2189
NM_002966	SEQ ID NO 847	Contig35896_RC	SEQ ID NO 2190
NM_002982	SEQ ID NO 849	Contig35976_RC	SEQ ID NO 2191
NM_002983	SEQ ID NO 850	Contig36042_RC	SEQ ID NO 2192
NM_002984	SEQ ID NO 851	Contig36081_RC	SEQ ID NO 2193
NM_002985	SEQ ID NO 852	Contig36152_RC	SEQ ID NO 2194
NM_002988	SEQ ID NO 853	Contig36193_RC	SEQ ID NO 2195
NM_002996	SEQ ID NO 854	Contig36312_RC	SEQ ID NO 2196
NM_002997	SEQ ID NO 855	Contig36323_RC	SEQ ID NO 2197
NM_002999	SEQ ID NO 856	Contig36339_RC	SEQ ID NO 2198
NM_003012	SEQ ID NO 857	Contig36647_RC	SEQ ID NO 2199
NM_003022	SEQ ID NO 858	Contig36744_RC	SEQ ID NO 2200
NM_003034	SEQ ID NO 859	Contig36761_RC	SEQ ID NO 2201
NM_003035	SEQ ID NO 860	Contig36879_RC	SEQ ID NO 2202
NM_003039	SEQ ID NO 861	Contig36900_RC	SEQ ID NO 2203

GenBank Accession Number	SEQ ID NO	GenBank Accession Number	SEQ ID NO
NM_003051	SEQ ID NO 862	Contig37015_RC	SEQ ID NO 2204
NM_003064	SEQ ID NO 863	Contig37024_RC	SEQ ID NO 2205
NM_003066	SEQ ID NO 864	Contig37072_RC	SEQ ID NO 2207
NM_003088	SEQ ID NO 865	Contig37140_RC	SEQ ID NO 2208
NM_003090	SEQ ID NO 866	Contig37141_RC	SEQ ID NO 2209
NM_003096	SEQ ID NO 867	Contig37204_RC	SEQ ID NO 2210
NM_003099	SEQ ID NO 868	Contig37281_RC	SEQ ID NO 2211
NM_003102	SEQ ID NO 869	Contig37287_RC	SEQ ID NO 2212
NM_003104	SEQ ID NO 870	Contig37439_RC	SEQ ID NO 2213
NM_003108	SEQ ID NO 871	Contig37562_RC	SEQ ID NO 2214
NM_003121	SEQ ID NO 873	Contig37571_RC	SEQ ID NO 2215
NM_003134	SEQ ID NO 874	Contig37598	SEQ ID NO 2216
NM_003137	SEQ ID NO 875	Contig37758_RC	SEQ ID NO 2217
NM_003144	SEQ ID NO 876	Contig37778_RC	SEQ ID NO 2218
NM_003146	SEQ ID NO 877	Contig37884_RC	SEQ ID NO 2219
NM_003149	SEQ ID NO 878	Contig37946_RC	SEQ ID NO 2220
NM_003151	SEQ ID NO 879	Contig38170_RC	SEQ ID NO 2221
NM_003157	SEQ ID NO 880	Contig38288_RC	SEQ ID NO 2223
NM_003158	SEQ ID NO 881	Contig38398_RC	SEQ ID NO 2224
NM_003165	SEQ ID NO 882	Contig38580_RC	SEQ ID NO 2226
NM_003172	SEQ ID NO 883	Contig38630_RC	SEQ ID NO 2227
NM_003177	SEQ ID NO 884	Contig38652_RC	SEQ ID NO 2228
NM_003197	SEQ ID NO 885	Contig38683_RC	SEQ ID NO 2229
NM_003202	SEQ ID NO 886	Contig38726_RC	SEQ ID NO 2230
NM_003213	SEQ ID NO 887	Contig38791_RC	SEQ ID NO 2231
NM_003217	SEQ ID NO 888	Contig38901_RC	SEQ ID NO 2232
NM_003225	SEQ ID NO 889	Contig38983_RC	SEQ ID NO 2233
NM_003226	SEQ ID NO 890	Contig39090_RC	SEQ ID NO 2234
NM_003236	SEQ ID NO 892	Contig39132_RC	SEQ ID NO 2235
NM_003239	SEQ ID NO 893	Contig39157_RC	SEQ ID NO 2236
NM_003248	SEQ ID NO 894	Contig39226_RC	SEQ ID NO 2237
NM_003255	SEQ ID NO 895	Contig39285_RC	SEQ ID NO 2238
NM_003258	SEQ ID NO 896	Contig39556_RC	SEQ ID NO 2239
NM_003264	SEQ ID NO 897	Contig39591_RC	SEQ ID NO 2240
NM_003283	SEQ ID NO 898	Contig39826_RC	SEQ ID NO 2241
NM_003318	SEQ ID NO 899	Contig39845_RC	SEQ ID NO 2242
NM_003329	SEQ ID NO 900	Contig39891_RC	SEQ ID NO 2243

GenBank Accession Number	SEQ ID NO	GenBank Accession Number	SEQ ID NO
NM_003332	SEQ ID NO 901	Contig39922_RC	SEQ ID NO 2244
NM_003358	SEQ ID NO 902	Contig39960_RC	SEQ ID NO 2245
NM_003359	SEQ ID NO 903	Contig40026_RC	SEQ ID NO 2246
NM_003360	SEQ ID NO 904	Contig40121_RC	SEQ ID NO 2247
NM_003368	SEQ ID NO 905	Contig40128_RC	SEQ ID NO 2248
NM_003376	SEQ ID NO 906	Contig40146	SEQ ID NO 2249
NM_003380	SEQ ID NO 907	Contig40208_RC	SEQ ID NO 2250
NM_003392	SEQ ID NO 908	Contig40212_RC	SEQ ID NO 2251
NM_003412	SEQ ID NO 909	Contig40238_RC	SEQ ID NO 2252
NM_003430	SEQ ID NO 910	Contig40434_RC	SEQ ID NO 2253
NM_003462	SEQ ID NO 911	Contig40446_RC	SEQ ID NO 2254
NM_003467	SEQ ID NO 912	Contig40500_RC	SEQ ID NO 2255
NM_003472	SEQ ID NO 913	Contig40573_RC	SEQ ID NO 2256
NM_003479	SEQ ID NO 914	Contig40813_RC	SEQ ID NO 2258
NM_003489	SEQ ID NO 915	Contig40816_RC	SEQ ID NO 2259
NM_003494	SEQ ID NO 916	Contig40845_RC	SEQ ID NO 2261
NM_003498	SEQ ID NO 917	Contig40889_RC	SEQ ID NO 2262
NM_003504	SEQ ID NO 919	Contig41035	SEQ ID NO 2263
NM_003508	SEQ ID NO 920	Contig41234_RC	SEQ ID NO 2264
NM_003510	SEQ ID NO 921	Contig41413_RC	SEQ ID NO 2266
NM_003512	SEQ ID NO 922	Contig41521_RC	SEQ ID NO 2267
NM_003528	SEQ ID NO 923	Contig41530_RC	SEQ ID NO 2268
NM_003544	SEQ ID NO 924	Contig41590	SEQ ID NO 2269
NM_003561	SEQ ID NO 925	Contig41618_RC	SEQ ID NO 2270
NM_003563	SEQ ID NO 926	Contig41624_RC	SEQ ID NO 2271
NM_003568	SEQ ID NO 927	Contig41635_RC	SEQ ID NO 2272
NM_003579	SEQ ID NO 928	Contig41676_RC	SEQ ID NO 2273
NM_003600	SEQ ID NO 929	Contig41689_RC	SEQ ID NO 2274
NM_003615	SEQ ID NO 931	Contig41804_RC	SEQ ID NO 2275
NM_003627	SEQ ID NO 932	Contig41887_RC	SEQ ID NO 2276
NM_003645	SEQ ID NO 935	Contig41905_RC	SEQ ID NO 2277
NM_003651	SEQ ID NO 936	Contig41954_RC	SEQ ID NO 2278
NM_003657	SEQ ID NO 937	Contig41983_RC	SEQ ID NO 2279
NM_003662	SEQ ID NO 938	Contig42006_RC	SEQ ID NO 2280
NM_003670	SEQ ID NO 939	Contig42014_RC	SEQ ID NO 2281
NM_003675	SEQ ID NO 940	Contig42036_RC	SEQ ID NO 2282
NM_003676	SEQ ID NO 941	Contig42041_RC	SEQ ID NO 2283

GenBank Accession Number	SEQ ID NO	GenBank Accession Number	SEQ ID NO
NM_003681	SEQ ID NO 942	Contig42139	SEQ ID NO 2284
NM_003683	SEQ ID NO 943	Contig42161_RC	SEQ ID NO 2285
NM_003686	SEQ ID NO 944	Contig42220_RC	SEQ ID NO 2286
NM_003689	SEQ ID NO 945	Contig42306_RC	SEQ ID NO 2287
NM_003714	SEQ ID NO 946	Contig42311_RC	SEQ ID NO 2288
NM_003720	SEQ ID NO 947	Contig42313_RC	SEQ ID NO 2289
NM_003726	SEQ ID NO 948	Contig42402_RC	SEQ ID NO 2290
NM_003729	SEQ ID NO 949	Contig42421_RC	SEQ ID NO 2291
NM_003740	SEQ ID NO 950	Contig42430_RC	SEQ ID NO 2292
NM_003772	SEQ ID NO 952	Contig42431_RC	SEQ ID NO 2293
NM_003791	SEQ ID NO 953	Contig42542_RC	SEQ ID NO 2294
NM_003793	SEQ ID NO 954	Contig42582	SEQ ID NO 2295
NM_003795	SEQ ID NO 955	Contig42631_RC	SEQ ID NO 2296
NM_003806	SEQ ID NO 956	Contig42751_RC	SEQ ID NO 2297
NM_003821	SEQ ID NO 957	Contig42759_RC	SEQ ID NO 2298
NM_003829	SEQ ID NO 958	Contig43054	SEQ ID NO 2299
NM_003831	SEQ ID NO 959	Contig43079_RC	SEQ ID NO 2300
NM_003862	SEQ ID NO 960	Contig43195_RC	SEQ ID NO 2301
NM_003866	SEQ ID NO 961	Contig43368_RC	SEQ ID NO 2302
NM_003875	SEQ ID NO 962	Contig43410_RC	SEQ ID NO 2303
NM_003878	SEQ ID NO 963	Contig43476_RC	SEQ ID NO 2304
NM_003894	SEQ ID NO 965	Contig43549_RC	SEQ ID NO 2305
NM_003897	SEQ ID NO 966	Contig43645_RC	SEQ ID NO 2306
NM_003904	SEQ ID NO 967	Contig43648_RC	SEQ ID NO 2307
NM_003929	SEQ ID NO 968	Contig43673_RC	SEQ ID NO 2308
NM_003933	SEQ ID NO 969	Contig43679_RC	SEQ ID NO 2309
NM_003937	SEQ ID NO 970	Contig43694_RC	SEQ ID NO 2310
NM_003940	SEQ ID NO 971	Contig43747_RC	SEQ ID NO 2311
NM_003942	SEQ ID NO 972	Contig43918_RC	SEQ ID NO 2312
NM_003944	SEQ ID NO 973	Contig43983_RC	SEQ ID NO 2313
NM_003953	SEQ ID NO 974	Contig44040_RC	SEQ ID NO 2314
NM_003954	SEQ ID NO 975	Contig44064_RC	SEQ ID NO 2315
NM_003975	SEQ ID NO 976	Contig44195_RC	SEQ ID NO 2316
NM_003981	SEQ ID NO 977	Contig44226_RC	SEQ ID NO 2317
NM_003982	SEQ ID NO 978	Contig44289_RC	SEQ ID NO 2320
NM_003986	SEQ ID NO 979	Contig44310_RC	SEQ ID NO 2321
NM_004003	SEQ ID NO 980	Contig44409	SEQ ID NO 2322

GenBank Accession Number	SEQ ID NO	GenBank Accession Number	SEQ ID NO
NM_004010	SEQ ID NO 981	Contig44413_RC	SEQ ID NO 2323
NM_004024	SEQ ID NO 982	Contig44451_RC	SEQ ID NO 2324
NM_004038	SEQ ID NO 983	Contig44585_RC	SEQ ID NO 2325
NM_004049	SEQ ID NO 984	Contig44656_RC	SEQ ID NO 2326
NM_004052	SEQ ID NO 985	Contig44703_RC	SEQ ID NO 2327
NM_004053	SEQ ID NO 986	Contig44708_RC	SEQ ID NO 2328
NM_004079	SEQ ID NO 987	Contig44757_RC	SEQ ID NO 2329
NM_004104	SEQ ID NO 988	Contig44829_RC	SEQ ID NO 2331
NM_004109	SEQ ID NO 989	Contig44870	SEQ ID NO 2332
NM_004110	SEQ ID NO 990	Contig44893_RC	SEQ ID NO 2333
NM_004120	SEQ ID NO 991	Contig44909_RC	SEQ ID NO 2334
NM_004131	SEQ ID NO 992	Contig44939_RC	SEQ ID NO 2335
NM_004143	SEQ ID NO 993	Contig45022_RC	SEQ ID NO 2336
NM_004154	SEQ ID NO 994	Contig45032_RC	SEQ ID NO 2337
NM_004170	SEQ ID NO 996	Contig45041_RC	SEQ ID NO 2338
NM_004172	SEQ ID NO 997	Contig45049_RC	SEQ ID NO 2339
NM_004176	SEQ ID NO 998	Contig45090_RC	SEQ ID NO 2340
NM_004180	SEQ ID NO 999	Contig45156_RC	SEQ ID NO 2341
NM_004181	SEQ ID NO 1000	Contig45316_RC	SEQ ID NO 2342
NM_004184	SEQ ID NO 1001	Contig45321	SEQ ID NO 2343
NM_004203	SEQ ID NO 1002	Contig45375_RC	SEQ ID NO 2345
NM_004207	SEQ ID NO 1003	Contig45443_RC	SEQ ID NO 2346
NM_004217	SEQ ID NO 1004	Contig45454_RC	SEQ ID NO 2347
NM_004219	SEQ ID NO 1005	Contig45537_RC	SEQ ID NO 2348
NM_004221	SEQ ID NO 1006	Contig45588_RC	SEQ ID NO 2349
NM_004233	SEQ ID NO 1007	Contig45708_RC	SEQ ID NO 2350
NM_004244	SEQ ID NO 1008	Contig45816_RC	SEQ ID NO 2351
NM_004252	SEQ ID NO 1009	Contig45847_RC	SEQ ID NO 2352
NM_004265	SEQ ID NO 1010	Contig45891_RC	SEQ ID NO 2353
NM_004267	SEQ ID NO 1011	Contig46056_RC	SEQ ID NO 2354
NM_004281	SEQ ID NO 1012	Contig46062_RC	SEQ ID NO 2355
NM_004289	SEQ ID NO 1013	Contig46075_RC	SEQ ID NO 2356
NM_004298	SEQ ID NO 1015	Contig46164_RC	SEQ ID NO 2357
NM_004301	SEQ ID NO 1016	Contig46218_RC	SEQ ID NO 2358
NM_004305	SEQ ID NO 1017	Contig46223_RC	SEQ ID NO 2359
NM_004311	SEQ ID NO 1018	Contig46244_RC	SEQ ID NO 2360
NM_004315	SEQ ID NO 1019	Contig46262_RC	SEQ ID NO 2361

GenBank Accession Number	SEQ ID NO	GenBank Accession Number	SEQ ID NO
NM_004323	SEQ ID NO 1020	Contig46362_RC	SEQ ID NO 2364
NM_004330	SEQ ID NO 1021	Contig46443_RC	SEQ ID NO 2365
NM_004336	SEQ ID NO 1022	Contig46553_RC	SEQ ID NO 2367
NM_004338	SEQ ID NO 1023	Contig46597_RC	SEQ ID NO 2368
NM_004350	SEQ ID NO 1024	Contig46653_RC	SEQ ID NO 2369
NM_004354	SEQ ID NO 1025	Contig46709_RC	SEQ ID NO 2370
NM_004358	SEQ ID NO 1026	Contig46777_RC	SEQ ID NO 2371
NM_004360	SEQ ID NO 1027	Contig46802_RC	SEQ ID NO 2372
NM_004362	SEQ ID NO 1028	Contig46890_RC	SEQ ID NO 2374
NM_004374	SEQ ID NO 1029	Contig46922_RC	SEQ ID NO 2375
NM_004378	SEQ ID NO 1030	Contig46934_RC	SEQ ID NO 2376
NM_004392	SEQ ID NO 1031	Contig46937_RC	SEQ ID NO 2377
NM_004395	SEQ ID NO 1032	Contig46991_RC	SEQ ID NO 2378
NM_004414	SEQ ID NO 1033	Contig47016_RC	SEQ ID NO 2379
NM_004418	SEQ ID NO 1034	Contig47045_RC	SEQ ID NO 2380
NM_004425	SEQ ID NO 1035	Contig47106_RC	SEQ ID NO 2381
NM_004431	SEQ ID NO 1036	Contig47146_RC	SEQ ID NO 2382
NM_004436	SEQ ID NO 1037	Contig47230_RC	SEQ ID NO 2383
NM_004438	SEQ ID NO 1038	Contig47405_RC	SEQ ID NO 2384
NM_004443	SEQ ID NO 1039	Contig47456_RC	SEQ ID NO 2385
NM_004446	SEQ ID NO 1040	Contig47465_RC	SEQ ID NO 2386
NM_004451	SEQ ID NO 1041	Contig47498_RC	SEQ ID NO 2387
NM_004454	SEQ ID NO 1042	Contig47578_RC	SEQ ID NO 2388
NM_004456	SEQ ID NO 1043	Contig47645_RC	SEQ ID NO 2389
NM_004458	SEQ ID NO 1044	Contig47680_RC	SEQ ID NO 2390
NM_004472	SEQ ID NO 1045	Contig47781_RC	SEQ ID NO 2391
NM_004480	SEQ ID NO 1046	Contig47814_RC	SEQ ID NO 2392
NM_004482	SEQ ID NO 1047	Contig48004_RC	SEQ ID NO 2393
NM_004494	SEQ ID NO 1048	Contig48043_RC	SEQ ID NO 2394
NM_004496	SEQ ID NO 1049	Contig48057_RC	SEQ ID NO 2395
NM_004503	SEQ ID NO 1050	Contig48076_RC	SEQ ID NO 2396
NM_004504	SEQ ID NO 1051	Contig48249_RC	SEQ ID NO 2397
NM_004515	SEQ ID NO 1052	Contig48263_RC	SEQ ID NO 2398
NM_004522	SEQ ID NO 1053	Contig48270_RC	SEQ ID NO 2399
NM_004523	SEQ ID NO 1054	Contig48328_RC	SEQ ID NO 2400
NM_004525	SEQ ID NO 1055	Contig48518_RC	SEQ ID NO 2401
NM_004556	SEQ ID NO 1056	Contig48572_RC	SEQ ID NO 2402

GenBank Accession Number	SEQ ID NO	GenBank Accession Number	SEQ ID NO
NM_004559	SEQ ID NO 1057	Contig48659_RC	SEQ ID NO 2403
NM_004569	SEQ ID NO 1058	Contig48722_RC	SEQ ID NO 2404
NM_004577	SEQ ID NO 1059	Contig48774_RC	SEQ ID NO 2405
NM_004585	SEQ ID NO 1060	Contig48776_RC	SEQ ID NO 2406
NM_004587	SEQ ID NO 1061	Contig48800_RC	SEQ ID NO 2407
NM_004594	SEQ ID NO 1062	Contig48806_RC	SEQ ID NO 2408
NM_004599	SEQ ID NO 1063	Contig48852_RC	SEQ ID NO 2409
NM_004633	SEQ ID NO 1066	Contig48900_RC	SEQ ID NO 2410
NM_004642	SEQ ID NO 1067	Contig48913_RC	SEQ ID NO 2411
NM_004648	SEQ ID NO 1068	Contig48970_RC	SEQ ID NO 2413
NM_004663	SEQ ID NO 1069	Contig49058_RC	SEQ ID NO 2414
NM_004664	SEQ ID NO 1070	Contig49063_RC	SEQ ID NO 2415
NM_004684	SEQ ID NO 1071	Contig49093	SEQ ID NO 2416
NM_004688	SEQ ID NO 1072	Contig49098_RC	SEQ ID NO 2417
NM_004694	SEQ ID NO 1073	Contig49169_RC	SEQ ID NO 2418
NM_004695	SEQ ID NO 1074	Contig49233_RC	SEQ ID NO 2419
NM_004701	SEQ ID NO 1075	Contig49270_RC	SEQ ID NO 2420
NM_004708	SEQ ID NO 1077	Contig49282_RC	SEQ ID NO 2421
NM_004711	SEQ ID NO 1078	Contig49289_RC	SEQ ID NO 2422
NM_004726	SEQ ID NO 1079	Contig49342_RC	SEQ ID NO 2423
NM_004750	SEQ ID NO 1081	Contig49344	SEQ ID NO 2424
NM_004761	SEQ ID NO 1082	Contig49388_RC	SEQ ID NO 2425
NM_004762	SEQ ID NO 1083	Contig49405_RC	SEQ ID NO 2426
NM_004780	SEQ ID NO 1085	Contig49445_RC	SEQ ID NO 2427
NM_004791	SEQ ID NO 1086	Contig49468_RC	SEQ ID NO 2428
NM_004798	SEQ ID NO 1087	Contig49509_RC	SEQ ID NO 2429
NM_004808	SEQ ID NO 1088	Contig49578_RC	SEQ ID NO 2431
NM_004811	SEQ ID NO 1089	Contig49581_RC	SEQ ID NO 2432
NM_004833	SEQ ID NO 1090	Contig49631_RC	SEQ ID NO 2433
NM_004835	SEQ ID NO 1091	Contig49673_RC	SEQ ID NO 2435
NM_004843	SEQ ID NO 1092	Contig49743_RC	SEQ ID NO 2436
NM_004847	SEQ ID NO 1093	Contig49790_RC	SEQ ID NO 2437
NM_004848	SEQ ID NO 1094	Contig49818_RC	SEQ ID NO 2438
NM_004864	SEQ ID NO 1095	Contig49849_RC	SEQ ID NO 2439
NM_004865	SEQ ID NO 1096	Contig49855	SEQ ID NO 2440
NM_004866	SEQ ID NO 1097	Contig49910_RC	SEQ ID NO 2441
NM_004877	SEQ ID NO 1098	Contig49948_RC	SEQ ID NO 2442

GenBank Accession Number	SEQ ID NO	GenBank Accession Number	SEQ ID NO
NM_004900	SEQ ID NO 1099	Contig50004_RC	SEQ ID NO 2443
NM_004906	SEQ ID NO 1100	Contig50094	SEQ ID NO 2444
NM_004910	SEQ ID NO 1101	Contig50120_RC	SEQ ID NO 2446
NM_004918	SEQ ID NO 1103	Contig50153_RC	SEQ ID NO 2447
NM_004923	SEQ ID NO 1104	Contig50189_RC	SEQ ID NO 2448
NM_004938	SEQ ID NO 1105	Contig50276_RC	SEQ ID NO 2449
NM_004951	SEQ ID NO 1106	Contig50288_RC	SEQ ID NO 2450
NM_004968	SEQ ID NO 1107	Contig50297_RC	SEQ ID NO 2451
NM_004994	SEQ ID NO 1108	Contig50391_RC	SEQ ID NO 2452
NM_004999	SEQ ID NO 1109	Contig50410	SEQ ID NO 2453
NM_005001	SEQ ID NO 1110	Contig50523_RC	SEQ ID NO 2454
NM_005002	SEQ ID NO 1111	Contig50529	SEQ ID NO 2455
NM_005012	SEQ ID NO 1112	Contig50588_RC	SEQ ID NO 2456
NM_005032	SEQ ID NO 1113	Contig50592	SEQ ID NO 2457
NM_005044	SEQ ID NO 1114	Contig50669_RC	SEQ ID NO 2458
NM_005046	SEQ ID NO 1115	Contig50719_RC	SEQ ID NO 2460
NM_005049	SEQ ID NO 1116	Contig50728_RC	SEQ ID NO 2461
NM_005067	SEQ ID NO 1117	Contig50731_RC	SEQ ID NO 2462
NM_005077	SEQ ID NO 1118	Contig50802_RC	SEQ ID NO 2463
NM_005080	SEQ ID NO 1119	Contig50822_RC	SEQ ID NO 2464
NM_005084	SEQ ID NO 1120	Contig50850_RC	SEQ ID NO 2466
NM_005130	SEQ ID NO 1122	Contig50860_RC	SEQ ID NO 2467
NM_005139	SEQ ID NO 1123	Contig50913_RC	SEQ ID NO 2468
NM_005168	SEQ ID NO 1125	Contig50950_RC	SEQ ID NO 2469
NM_005190	SEQ ID NO 1126	Contig51066_RC	SEQ ID NO 2470
NM_005196	SEQ ID NO 1127	Contig51105_RC	SEQ ID NO 2472
NM_005213	SEQ ID NO 1128	Contig51117_RC	SEQ ID NO 2473
NM_005218	SEQ ID NO 1129	Contig51196_RC	SEQ ID NO 2474
NM_005235	SEQ ID NO 1130	Contig51235_RC	SEQ ID NO 2475
NM_005245	SEQ ID NO 1131	Contig51254_RC	SEQ ID NO 2476
NM_005249	SEQ ID NO 1132	Contig51352_RC	SEQ ID NO 2477
NM_005257	SEQ ID NO 1133	Contig51369_RC	SEQ ID NO 2478
NM_005264	SEQ ID NO 1134	Contig51392_RC	SEQ ID NO 2479
NM_005271	SEQ ID NO 1135	Contig51403_RC	SEQ ID NO 2480
NM_005314	SEQ ID NO 1136	Contig51685_RC	SEQ ID NO 2483
NM_005321	SEQ ID NO 1137	Contig51726_RC	SEQ ID NO 2484
NM_005322	SEQ ID NO 1138	Contig51742_RC	SEQ ID NO 2485

GenBank Accession Number	SEQ ID NO	GenBank Accession Number	SEQ ID NO
NM_005325	SEQ ID NO 1139	Contig51749_RC	SEQ ID NO 2486
NM_005326	SEQ ID NO 1140	Contig51775_RC	SEQ ID NO 2487
NM_005335	SEQ ID NO 1141	Contig51800	SEQ ID NO 2488
NM_005337	SEQ ID NO 1142	Contig51809_RC	SEQ ID NO 2489
NM_005342	SEQ ID NO 1143	Contig51821_RC	SEQ ID NO 2490
NM_005345	SEQ ID NO 1144	Contig51888_RC	SEQ ID NO 2491
NM_005357	SEQ ID NO 1145	Contig51953_RC	SEQ ID NO 2493
NM_005375	SEQ ID NO 1146	Contig51967_RC	SEQ ID NO 2495
NM_005391	SEQ ID NO 1147	Contig51981_RC	SEQ ID NO 2496
NM_005408	SEQ ID NO 1148	Contig51994_RC	SEQ ID NO 2497
NM_005409	SEQ ID NO 1149	Contig52082_RC	SEQ ID NO 2498
NM_005410	SEQ ID NO 1150	Contig52094_RC	SEQ ID NO 2499
NM_005426	SEQ ID NO 1151	Contig52320	SEQ ID NO 2500
NM_005433	SEQ ID NO 1152	Contig52398_RC	SEQ ID NO 2501
NM_005441	SEQ ID NO 1153	Contig52425_RC	SEQ ID NO 2503
NM_005443	SEQ ID NO 1154	Contig52482_RC	SEQ ID NO 2504
NM_005483	SEQ ID NO 1155	Contig52543_RC	SEQ ID NO 2505
NM_005486	SEQ ID NO 1156	Contig52553_RC	SEQ ID NO 2506
NM_005496	SEQ ID NO 1157	Contig52579_RC	SEQ ID NO 2507
NM_005498	SEQ ID NO 1158	Contig52603_RC	SEQ ID NO 2508
NM_005499	SEQ ID NO 1159	Contig52639_RC	SEQ ID NO 2509
NM_005514	SEQ ID NO 1160	Contig52641_RC	SEQ ID NO 2510
NM_005531	SEQ ID NO 1162	Contig52684	SEQ ID NO 2511
NM_005538	SEQ ID NO 1163	Contig52705_RC	SEQ ID NO 2512
NM_005541	SEQ ID NO 1164	Contig52720_RC	SEQ ID NO 2513
NM_005544	SEQ ID NO 1165	Contig52722_RC	SEQ ID NO 2514
NM_005548	SEQ ID NO 1166	Contig52723_RC	SEQ ID NO 2515
NM_005554	SEQ ID NO 1167	Contig52740_RC	SEQ ID NO 2516
NM_005555	SEQ ID NO 1168	Contig52779_RC	SEQ ID NO 2517
NM_005556	SEQ ID NO 1169	Contig52957_RC	SEQ ID NO 2518
NM_005557	SEQ ID NO 1170	Contig52994_RC	SEQ ID NO 2519
NM_005558	SEQ ID NO 1171	Contig53022_RC	SEQ ID NO 2520
NM_005562	SEQ ID NO 1172	Contig53038_RC	SEQ ID NO 2521
NM_005563	SEQ ID NO 1173	Contig53047_RC	SEQ ID NO 2522
NM_005565	SEQ ID NO 1174	Contig53130	SEQ ID NO 2523
NM_005566	SEQ ID NO 1175	Contig53183_RC	SEQ ID NO 2524
NM_005572	SEQ ID NO 1176	Contig53242_RC	SEQ ID NO 2526

GenBank Accession Number	SEQ ID NO	GenBank Accession Number	SEQ ID NO
NM_005582	SEQ ID NO 1177	Contig53248_RC	SEQ ID NO 2527
NM_005608	SEQ ID NO 1178	Contig53260_RC	SEQ ID NO 2528
NM_005614	SEQ ID NO 1179	Contig53296_RC	SEQ ID NO 2531
NM_005617	SEQ ID NO 1180	Contig53307_RC	SEQ ID NO 2532
NM_005620	SEQ ID NO 1181	Contig53314_RC	SEQ ID NO 2533
NM_005625	SEQ ID NO 1182	Contig53401_RC	SEQ ID NO 2534
NM_005651	SEQ ID NO 1183	Contig53550_RC	SEQ ID NO 2535
NM_005658	SEQ ID NO 1184	Contig53551_RC	SEQ ID NO 2536
NM_005659	SEQ ID NO 1185	Contig53598_RC	SEQ ID NO 2537
NM_005667	SEQ ID NO 1186	Contig53646_RC	SEQ ID NO 2538
NM_005686	SEQ ID NO 1187	Contig53658_RC	SEQ ID NO 2539
NM_005690	SEQ ID NO 1188	Contig53698_RC	SEQ ID NO 2540
NM_005720	SEQ ID NO 1190	Contig53719_RC	SEQ ID NO 2541
NM_005727	SEQ ID NO 1191	Contig53742_RC	SEQ ID NO 2542
NM_005733	SEQ ID NO 1192	Contig53757_RC	SEQ ID NO 2543
NM_005737	SEQ ID NO 1193	Contig53870_RC	SEQ ID NO 2544
NM_005742	SEQ ID NO 1194	Contig53952_RC	SEQ ID NO 2546
NM_005746	SEQ ID NO 1195	Contig53962_RC	SEQ ID NO 2547
NM_005749	SEQ ID NO 1196	Contig53968_RC	SEQ ID NO 2548
NM_005760	SEQ ID NO 1197	Contig54113_RC	SEQ ID NO 2549
NM_005764	SEQ ID NO 1198	Contig54142_RC	SEQ ID NO 2550
NM_005794	SEQ ID NO 1199	Contig54232_RC	SEQ ID NO 2551
NM_005796	SEQ ID NO 1200	Contig54242_RC	SEQ ID NO 2552
NM_005804	SEQ ID NO 1201	Contig54260_RC	SEQ ID NO 2553
NM_005813	SEQ ID NO 1202	Contig54263_RC	SEQ ID NO 2554
NM_005824	SEQ ID NO 1203	Contig54295_RC	SEQ ID NO 2555
NM_005825	SEQ ID NO 1204	Contig54318_RC	SEQ ID NO 2556
NM_005849	SEQ ID NO 1205	Contig54325_RC	SEQ ID NO 2557
NM_005853	SEQ ID NO 1206	Contig54389_RC	SEQ ID NO 2558
NM_005855	SEQ ID NO 1207	Contig54394_RC	SEQ ID NO 2559
NM_005864	SEQ ID NO 1208	Contig54414_RC	SEQ ID NO 2560
NM_005874	SEQ ID NO 1209	Contig54425	SEQ ID NO 2561
NM_005876	SEQ ID NO 1210	Contig54477_RC	SEQ ID NO 2562
NM_005880	SEQ ID NO 1211	Contig54503_RC	SEQ ID NO 2563
NM_005891	SEQ ID NO 1212	Contig54534_RC	SEQ ID NO 2564
NM_005892	SEQ ID NO 1213	Contig54560_RC	SEQ ID NO 2566
NM_005899	SEQ ID NO 1214	Contig54581_RC	SEQ ID NO 2567

GenBank Accession Number	SEQ ID NO	GenBank Accession Number	SEQ ID NO
NM_005915	SEQ ID NO 1215	Contig54609_RC	SEQ ID NO 2568
NM_005919	SEQ ID NO 1216	Contig54666_RC	SEQ ID NO 2569
NM_005923	SEQ ID NO 1217	Contig54667_RC	SEQ ID NO 2570
NM_005928	SEQ ID NO 1218	Contig54726_RC	SEQ ID NO 2571
NM_005932	SEQ ID NO 1219	Contig54742_RC	SEQ ID NO 2572
NM_005935	SEQ ID NO 1220	Contig54745_RC	SEQ ID NO 2573
NM_005945	SEQ ID NO 1221	Contig54757_RC	SEQ ID NO 2574
NM_005953	SEQ ID NO 1222	Contig54761_RC	SEQ ID NO 2575
NM_005978	SEQ ID NO 1223	Contig54813_RC	SEQ ID NO 2576
NM_005990	SEQ ID NO 1224	Contig54867_RC	SEQ ID NO 2577
NM_006002	SEQ ID NO 1225	Contig54895_RC	SEQ ID NO 2578
NM_006004	SEQ ID NO 1226	Contig54898_RC	SEQ ID NO 2579
NM_006005	SEQ ID NO 1227	Contig54913_RC	SEQ ID NO 2580
NM_006006	SEQ ID NO 1228	Contig54965_RC	SEQ ID NO 2582
NM_006017	SEQ ID NO 1229	Contig54968_RC	SEQ ID NO 2583
NM_006018	SEQ ID NO 1230	Contig55069_RC	SEQ ID NO 2584
NM_006023	SEQ ID NO 1231	Contig55181_RC	SEQ ID NO 2585
NM_006027	SEQ ID NO 1232	Contig55188_RC	SEQ ID NO 2586
NM_006029	SEQ ID NO 1233	Contig55221_RC	SEQ ID NO 2587
NM_006033	SEQ ID NO 1234	Contig55254_RC	SEQ ID NO 2588
NM_006051	SEQ ID NO 1235	Contig55265_RC	SEQ ID NO 2589
NM_006055	SEQ ID NO 1236	Contig55377_RC	SEQ ID NO 2591
NM_006074	SEQ ID NO 1237	Contig55397_RC	SEQ ID NO 2592
NM_006086	SEQ ID NO 1238	Contig55448_RC	SEQ ID NO 2593
NM_006087	SEQ ID NO 1239	Contig55468_RC	SEQ ID NO 2594
NM_006096	SEQ ID NO 1240	Contig55500_RC	SEQ ID NO 2595
NM_006101	SEQ ID NO 1241	Contig55538_RC	SEQ ID NO 2596
NM_006103	SEQ ID NO 1242	Contig55558_RC	SEQ ID NO 2597
NM_006111	SEQ ID NO 1243	Contig55606_RC	SEQ ID NO 2598
NM_006113	SEQ ID NO 1244	Contig55674_RC	SEQ ID NO 2599
NM_006115	SEQ ID NO 1245	Contig55725_RC	SEQ ID NO 2600
NM_006117	SEQ ID NO 1246	Contig55728_RC	SEQ ID NO 2601
NM_006142	SEQ ID NO 1247	Contig55756_RC	SEQ ID NO 2602
NM_006144	SEQ ID NO 1248	Contig55769_RC	SEQ ID NO 2603
NM_006148	SEQ ID NO 1249	Contig55771_RC	SEQ ID NO 2605
NM_006153	SEQ ID NO 1250	Contig55813_RC	SEQ ID NO 2607
NM_006159	SEQ ID NO 1251	Contig55829_RC	SEQ ID NO 2608

GenBank Accession Number	SEQ ID NO	GenBank Accession Number	SEQ ID NO
NM_006170	SEQ ID NO 1252	Contig55852_RC	SEQ ID NO 2609
NM_006197	SEQ ID NO 1253	Contig55883_RC	SEQ ID NO 2610
NM_006224	SEQ ID NO 1255	Contig55920_RC	SEQ ID NO 2611
NM_006227	SEQ ID NO 1256	Contig55940_RC	SEQ ID NO 2612
NM_006235	SEQ ID NO 1257	Contig55950_RC	SEQ ID NO 2613
NM_006243	SEQ ID NO 1258	Contig55991_RC	SEQ ID NO 2614
NM_006264	SEQ ID NO 1259	Contig55997_RC	SEQ ID NO 2615
NM_006271	SEQ ID NO 1261	Contig56023_RC	SEQ ID NO 2616
NM_006274	SEQ ID NO 1262	Contig56030_RC	SEQ ID NO 2617
NM_006290	SEQ ID NO 1265	Contig56093_RC	SEQ ID NO 2618
NM_006291	SEQ ID NO 1266	Contig56205_RC	SEQ ID NO 2621
NM_006296	SEQ ID NO 1267	Contig56270_RC	SEQ ID NO 2622
NM_006304	SEQ ID NO 1268	Contig56276_RC	SEQ ID NO 2623
NM_006314	SEQ ID NO 1269	Contig56291_RC	SEQ ID NO 2624
NM_006332	SEQ ID NO 1270	Contig56298_RC	SEQ ID NO 2625
NM_006357	SEQ ID NO 1271	Contig56307	SEQ ID NO 2627
NM_006366	SEQ ID NO 1272	Contig56390_RC	SEQ ID NO 2628
NM_006372	SEQ ID NO 1273	Contig56434_RC	SEQ ID NO 2629
NM_006377	SEQ ID NO 1274	Contig56457_RC	SEQ ID NO 2630
NM_006378	SEQ ID NO 1275	Contig56534_RC	SEQ ID NO 2631
NM_006383	SEQ ID NO 1276	Contig56670_RC	SEQ ID NO 2632
NM_006389	SEQ ID NO 1277	Contig56678_RC	SEQ ID NO 2633
NM_006393	SEQ ID NO 1278	Contig56742_RC	SEQ ID NO 2634
NM_006398	SEQ ID NO 1279	Contig56759_RC	SEQ ID NO 2635
NM_006406	SEQ ID NO 1280	Contig56765_RC	SEQ ID NO 2636
NM_006408	SEQ ID NO 1281	Contig56843_RC	SEQ ID NO 2637
NM_006410	SEQ ID NO 1282	Contig57011_RC	SEQ ID NO 2638
NM_006414	SEQ ID NO 1283	Contig57023_RC	SEQ ID NO 2639
NM_006417	SEQ ID NO 1284	Contig57057_RC	SEQ ID NO 2640
NM_006430	SEQ ID NO 1285	Contig57076_RC	SEQ ID NO 2641
NM_006460	SEQ ID NO 1286	Contig57081_RC	SEQ ID NO 2642
NM_006461	SEQ ID NO 1287	Contig57091_RC	SEQ ID NO 2643
NM_006469	SEQ ID NO 1288	Contig57138_RC	SEQ ID NO 2644
NM_006470	SEQ ID NO 1289	Contig57173_RC	SEQ ID NO 2645
NM_006491	SEQ ID NO 1290	Contig57230_RC	SEQ ID NO 2646
NM_006495	SEQ ID NO 1291	Contig57258_RC	SEQ ID NO 2647
NM_006500	SEQ ID NO 1292	Contig57270_RC	SEQ ID NO 2648

GenBank Accession Number	SEQ ID NO	GenBank Accession Number	SEQ ID NO
NM_006509	SEQ ID NO 1293	Contig57272_RC	SEQ ID NO 2649
NM_006516	SEQ ID NO 1294	Contig57344_RC	SEQ ID NO 2650
NM_006533	SEQ ID NO 1295	Contig57430_RC	SEQ ID NO 2651
NM_006551	SEQ ID NO 1296	Contig57458_RC	SEQ ID NO 2652
NM_006556	SEQ ID NO 1297	Contig57493_RC	SEQ ID NO 2653
NM_006558	SEQ ID NO 1298	Contig57584_RC	SEQ ID NO 2654
NM_006564	SEQ ID NO 1299	Contig57595	SEQ ID NO 2655
NM_006573	SEQ ID NO 1300	Contig57602_RC	SEQ ID NO 2656
NM_006607	SEQ ID NO 1301	Contig57609_RC	SEQ ID NO 2657
NM_006622	SEQ ID NO 1302	Contig57610_RC	SEQ ID NO 2658
NM_006623	SEQ ID NO 1303	Contig57644_RC	SEQ ID NO 2659
NM_006636	SEQ ID NO 1304	Contig57725_RC	SEQ ID NO 2660
NM_006670	SEQ ID NO 1305	Contig57739_RC	SEQ ID NO 2661
NM_006681	SEQ ID NO 1306	Contig57825_RC	SEQ ID NO 2662
NM_006682	SEQ ID NO 1307	Contig57864_RC	SEQ ID NO 2663
NM_006696	SEQ ID NO 1308	Contig57940_RC	SEQ ID NO 2664
NM_006698	SEQ ID NO 1309	Contig58260_RC	SEQ ID NO 2665
NM_006705	SEQ ID NO 1310	Contig58272_RC	SEQ ID NO 2666
NM_006739	SEQ ID NO 1311	Contig58301_RC	SEQ ID NO 2667
NM_006748	SEQ ID NO 1312	Contig58368_RC	SEQ ID NO 2668
NM_006759	SEQ ID NO 1313	Contig58471_RC	SEQ ID NO 2669
NM_006762	SEQ ID NO 1314	Contig58755_RC	SEQ ID NO 2671
NM_006763	SEQ ID NO 1315	Contig59120_RC	SEQ ID NO 2672
NM_006769	SEQ ID NO 1316	Contig60157_RC	SEQ ID NO 2673
NM_006770	SEQ ID NO 1317	Contig60864_RC	SEQ ID NO 2676
NM_006780	SEQ ID NO 1318	Contig61254_RC	SEQ ID NO 2677
NM_006787	SEQ ID NO 1319	Contig61815	SEQ ID NO 2678
NM_006806	SEQ ID NO 1320	Contig61975	SEQ ID NO 2679
NM_006813	SEQ ID NO 1321	Contig62306	SEQ ID NO 2680
NM_006825	SEQ ID NO 1322	Contig62568_RC	SEQ ID NO 2681
NM_006826	SEQ ID NO 1323	Contig62922_RC	SEQ ID NO 2682
NM_006829	SEQ ID NO 1324	Contig62964_RC	SEQ ID NO 2683
NM_006834	SEQ ID NO 1325	Contig63520_RC	SEQ ID NO 2685
NM_006835	SEQ ID NO 1326	Contig63649_RC	SEQ ID NO 2686
NM_006840	SEQ ID NO 1327	Contig63683_RC	SEQ ID NO 2687
NM_006845	SEQ ID NO 1328	Contig63748_RC	SEQ ID NO 2688
NM_006847	SEQ ID NO 1329	Contig64502	SEQ ID NO 2689

GenBank Accession Number	SEQ ID NO	GenBank Accession Number	SEQ ID NO
NM_006851	SEQ ID NO 1330	Contig64688	SEQ ID NO 2690
NM_006855	SEQ ID NO 1331	Contig64775_RC	SEQ ID NO 2691
NM_006864	SEQ ID NO 1332	Contig65227	SEQ ID NO 2692
NM_006868	SEQ ID NO 1333	Contig65663	SEQ ID NO 2693
NM_006875	SEQ ID NO 1334	Contig65785_RC	SEQ ID NO 2694
NM_006889	SEQ ID NO 1336	Contig65900	SEQ ID NO 2695
NM_006892	SEQ ID NO 1337	Contig66219_RC	SEQ ID NO 2696
NM_006912	SEQ ID NO 1338	Contig66705_RC	SEQ ID NO 2697
NM_006931	SEQ ID NO 1341	Contig66759_RC	SEQ ID NO 2698
NM_006941	SEQ ID NO 1342	Contig67182_RC	SEQ ID NO 2699
NM_006943	SEQ ID NO 1343		

Table 2. 550 preferred ER status markers drawn from Table 1.

Identifier	Correlation	Name	Description
NM_002051	0.763977	GATA3	GATA-binding protein 3
AB020689	0.753592	KIAA0882	KIAA0882 protein
NM_001218	0.753225	CA12	carbonic anhydrase XII
NM_000125	0.748421	ESR1	estrogen receptor 1
Contig56678_RC	0.747816		ESTs
NM_004496	0.729116	HNF3A	hepatocyte nuclear factor 3, alpha
NM_017732	0.713398	FLJ20262	hypothetical protein FLJ20262
NM_006806	-0.712678	BTG3	BTG family, member 3
Contig56390_RC	0.705940		ESTs
Contig37571_RC	0.704468		ESTs
NM_004559	-0.701617	NSEP1	nuclease sensitive element binding protein 1
Contig50153_RC	-0.696652		ESTs, Weakly similar to LKHU proteoglycan link protein precursor [H.sapiens]
NM_012155	0.694332	EMAP-2	microtubule-associated protein like echinoderm EMAP
Contig237_RC	0.687485	FLJ21127	hypothetical protein FLJ21127
NM_019063	-0.686064	C2ORF2	chromosome 2 open reading frame 2
NM_012219	-0.680900	MRAS	muscle RAS oncogene homolog
NM_001982	0.676114	ERBB3	v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 3
NM_006623	-0.675090	PHGDH	phosphoglycerate dehydrogenase
NM_000636	-0.674282	SOD2	superoxide dismutase 2, mitochondrial
NM_006017	-0.670353	PROML1	prominin (mouse)-like 1
Contig57940_RC	0.667915	MAP-1	MAP-1 protein
Contig46934_RC	0.666908		ESTs, Weakly similar to JE0350 Anterior gradient-2 [H.sapiens]
NM_005080	0.665772	XBP1	X-box binding protein 1
NM_014246	0.665725	CELSR1	cadherin, EGF LAG seven-pass G-type receptor 1, flamingo (Drosophila) homolog

Identifier	Correlation	Name	Description
Contig54667_RC	-0.663727		Human DNA sequence from clone RP1-187J11 on chromosome 6q11.1-22.33. Contains the gene for a novel protein similar to S. pombe and S. cerevisiae predicted proteins, the gene for a novel protein similar to protein kinase C inhibitors, the 3' end of the gene for a novel protein similar to Drosophila L82 and predicted worm proteins, ESTs, STSs, GSSs and two putative CpG islands
Contig51994_RC	0.663715		ESTs, Weakly similar to B0416.1 [C.elegans]
NM_016337	0.663006	RNB6	RNB6
NM_015640	-0.660165	PAI-RBP1	PAI-1 mRNA-binding protein
X07834	-0.657798	SOD2	superoxide dismutase 2, mitochondrial
NM_012319	0.657666	LIV-1	LIV-1 protein, estrogen regulated
Contig41887_RC	0.656042		ESTs, Weakly similar to Homolog of rat Zymogen granule membrane protein [H.sapiens]
NM_003462	0.655349	P28	dynein, axonemal, light intermediate polypeptide
Contig58301_RC	0.654268		Homo sapiens mRNA; cDNA DKFZp667D095 (from clone DKFZp667D095)
NM_005375	0.653783	MYB	v-myb avian myeloblastosis viral oncogene homolog
NM_017447	-0.652445	YG81	hypothetical protein LOC54149
Contig924_RC	-0.650658		ESTs
M55914	-0.650181	MPB1	MYC promoter-binding protein 1
NM_006004	-0.649819	UQCRH	ubiquinol-cytochrome c reductase hinge protein
NM_000964	0.649072	RARA	retinoic acid receptor, alpha
NM_013301	0.647583	HSU79303	protein predicted by clone 23882
AB023211	-0.647403	PDI2	peptidyl arginine deiminase, type II
NM_016629	-0.646412	LOC51323	hypothetical protein
K02403	0.645532	C4A	complement component 4A
NM_016405	-0.642201	HSU93243	Ubc6p homolog
Contig46597_RC	0.641733		ESTs
Contig55377_RC	0.640310		ESTs
NM_001207	0.637800	BTF3	basic transcription factor 3

Identifier	Correlation	Name	Description
NM_018166	0.636422	FLJ10647	hypothetical protein FLJ10647
AL110202	-0.635398		Homo sapiens mRNA; cDNA DKFZp586I2022 (from clone DKFZp586I2022)
AL133105	-0.635201	DKFZp434F2322	hypothetical protein DKFZp434F2322
NM_016839	-0.635169	RBMS1	RNA binding motif, single stranded interacting protein 1
Contig53130	-0.634812		ESTs, Weakly similar to hyperpolarization-activated cyclic nucleotide-gated channel hHCN2 [H.sapiens]
NM_018014	-0.634460	BCL11A	B-cell CLL/lymphoma 11A (zinc finger protein)
NM_006769	-0.632197	LMO4	LIM domain only 4
U92544	0.631170	JCL-1	hepatocellular carcinoma associated protein; breast cancer associated gene 1
Contig49233_RC	-0.631047		Homo sapiens, Similar to nuclear receptor binding factor 2, clone IMAGE:3463191, mRNA, partial cds
AL133033	0.629690	KIAA1025	KIAA1025 protein
AL049265	0.629414		Homo sapiens mRNA; cDNA DKFZp564F053 (from clone DKFZp564F053)
NM_018728	0.627989	MYO5C	myosin 5C
NM_004780	0.627856	TCEAL1	transcription elongation factor A (SII)-like 1
Contig760_RC	0.627132		ESTs
Contig399_RC	0.626543	FLJ12538	hypothetical protein FLJ12538 similar to ras-related protein RAB17
M83822	0.625092	CDC4L	cell division cycle 4-like
NM_001255	-0.625089	CDC20	CDC20 (cell division cycle 20, S. cerevisiae, homolog)
NM_006739	-0.624903	MCM5	minichromosome maintenance deficient (S. cerevisiae) 5 (cell division cycle 46)
NM_002888	-0.624664	RARRES1	retinoic acid receptor responder (tazarotene induced) 1
NM_003197	0.623850	TCEB1L	transcription elongation factor B (SIII), polypeptide 1-like
NM_006787	0.623625	JCL-1	hepatocellular carcinoma associated protein; breast cancer associated gene 1
Contig49342_RC	0.622179		ESTs

Identifier	Correlation	Name	Description
AL133619	0.621719		Homo sapiens mRNA; cDNA DKFZp434E2321 (from clone DKFZp434E2321); partial cds
AL133622	0.621577	KIAA0876	KIAA0876 protein
NM_004648	-0.621532	PTPNS1	protein tyrosine phosphatase, non-receptor type substrate 1
NM_001793	-0.621530	CDH3	cadherin 3, type 1, P-cadherin (placental)
NM_003217	0.620915	TEGT	testis enhanced gene transcript (BAX inhibitor 1)
NM_001551	0.620832	IGBP1	immunoglobulin (CD79A) binding protein 1
NM_002539	-0.620683	ODC1	ornithine decarboxylase 1
Contig55997_RC	-0.619932		ESTs
NM_000633	0.619547	BCL2	B-cell CLL/lymphoma 2
NM_016267	-0.619096	TONDU	TONDU
Contig3659_RC	0.618048	FLJ21174	hypothetical protein FLJ21174
NM_000191	0.617250	HMGCL	3-hydroxymethyl-3-methylglutaryl-Coenzyme A lyase (hydroxymethylglutaricaciduria)
NM_001267	0.616890	CHAD	chondroadherin
Contig39090_RC	0.616385		ESTs
AF055270	-0.616268	HSSG1	heat-shock suppressed protein 1
Contig43054	0.616015	FLJ21603	hypothetical protein FLJ21603
NM_001428	-0.615855	ENO1	enolase 1, (alpha)
Contig51369_RC	0.615466		ESTs
Contig36647_RC	0.615310	GFRA1	GDNF family receptor alpha 1
NM_014096	-0.614832	PRO1659	PRO1659 protein
NM_015937	0.614735	LOC51604	CGI-06 protein
Contig49790_RC	-0.614463		ESTs
NM_006759	-0.614279	UGP2	UDP-glucose pyrophosphorylase 2
Contig53598_RC	-0.613787	FLJ11413	hypothetical protein FLJ11413
AF113132	-0.613561	PSA	phosphoserine aminotransferase
AK000004	0.613001		Homo sapiens mRNA for FLJ00004 protein, partial cds
Contig52543_RC	0.612960		Homo sapiens cDNA FLJ13945 fis, clone Y79AA1000969
AB032966	-0.611917	KIAA1140	KIAA1140 protein
AL080192	0.611544		Homo sapiens cDNA: FLJ21238 fis, clone COL01115
X56807	-0.610654	DSC2	desmocollin 2

Identifier	Correlation	Name	Description
Contig30390_RC	0.609614		ESTs
AL137362	0.609121	FLJ22237	hypothetical protein FLJ22237
NM_014211	-0.608585	GABRP	gamma-aminobutyric acid (GABA) A receptor, pi
NM_006696	0.608474	SMAP	thyroid hormone receptor coactivating protein
Contig45588_RC	-0.608273		Homo sapiens cDNA: FLJ22610 fis, clone HSI04930
NM_003358	0.608244	UGCG	UDP-glucose ceramide glucosyltransferase
NM_006153	-0.608129	NCK1	NCK adaptor protein 1
NM_001453	-0.606939	FOXC1	forkhead box C1
Contig54666_RC	0.606475		oy65e02.x1 NCI_CGAP_CLL1 Homo sapiens cDNA clone IMAGE:1670714 3' similar to TR:Q29168 Q29168 UNKNOWN PROTEIN ;, mRNA sequence.
NM_005945	-0.605945	MPB1	MYC promoter-binding protein 1
Contig55725_RC	-0.605841		ESTs, Moderately similar to T50635 hypothetical protein DKFZp762L0311.1 [H.sapiens]
Contig37015_RC	-0.605780		ESTs, Weakly similar to UAS3_HUMAN UBASH3A PROTEIN [H.sapiens]
AL157480	-0.604362	SH3BP1	SH3-domain binding protein 1
NM_005325	-0.604310	H1F1	H1 histone family, member 1
NM_001446	-0.604061	FABP7	fatty acid binding protein 7, brain
Contig263_RC	0.603318		Homo sapiens cDNA: FLJ23000 fis, clone LNG00194
Contig8347_RC	-0.603311		ESTs
NM_002988	-0.603279	SCYA18	small inducible cytokine subfamily A (Cys-Cys), member 18, pulmonary and activation-regulated
AF111849	0.603157	HELO1	homolog of yeast long chain polyunsaturated fatty acid elongation enzyme 2
NM_014700	0.603042	KIAA0665	KIAA0665 gene product
NM_001814	-0.602988	CTSC	cathepsin C
AF116682	-0.602350	PRO2013	hypothetical protein PRO2013
AB037836	0.602024	KIAA1415	KIAA1415 protein
AB002301	0.602005	KIAA0303	KIAA0303 protein

Identifier	Correlation	Name	Description
NM_002996	-0.601841	SCYD1	small inducible cytokine subfamily D (Cys-X3-Cys), member 1 (fractalkine, neurotactin)
NM_018410	-0.601765	DKFZp762E1312	hypothetical protein DKFZp762E1312
Contig49581_RC	-0.601571	KIAA1350	KIAA1350 protein
NM_003088	-0.601458	SNL	singed (Drosophila)-like (sea urchin fascin homolog like)
Contig47045_RC	0.601088		ESTs, Weakly similar to DP1_HUMAN POLYPOSIS LOCUS PROTEIN 1 [H.sapiens]
NM_001806	-0.600954	CEBPG	CCAAT/enhancer binding protein (C/EBP), gamma
NM_004374	0.600766	COX6C	cytochrome c oxidase subunit VIc
Contig52641_RC	0.600132		ESTs, Weakly similar to CENB MOUSE MAJOR CENTROMERE AUTOANTIGEN B [M.musculus]
NM_000100	-0.600127	CSTB	cystatin B (stefin B)
NM_002250	-0.600004	KCNN4	potassium intermediate/small conductance calcium-activated channel, subfamily N, member 4
AB033035	-0.599423	KIAA1209	KIAA1209 protein
Contig53968_RC	0.599077		ESTs
NM_002300	-0.598246	LDHB	lactate dehydrogenase B
NM_000507	0.598110	FBP1	fructose-1,6-bisphosphatase 1
NM_002053	-0.597756	GBP1	guanylate binding protein 1, interferon-inducible, 67kD
AB007883	0.597043	KIAA0423	KIAA0423 protein
NM_004900	-0.597010	DJ742C19.2	phorbolin (similar to apolipoprotein B mRNA editing protein)
NM_004480	0.596321	FUT8	fucosyltransferase 8 (alpha (1,6) fucosyltransferase)
Contig35896_RC	0.596281		ESTs
NM_020974	0.595173	CEGP1	CEGP1 protein
NM_000662	0.595114	NAT1	N-acetyltransferase 1 (arylamine N-acetyltransferase)
NM_006113	0.595017	VAV3	vav 3 oncogene
NM_014865	-0.594928	KIAA0159	chromosome condensation-related SMC-associated protein 1
Contig55538_RC	-0.594573	BA395L14.2	hypothetical protein bA395L14.2
NM_016056	0.594084	LOC51643	CGI-119 protein

Identifier	Correlation	Name	Description
NM_003579	-0.594063	RAD54L	RAD54 (S.cerevisiae)-like
NM_014214	-0.593860	IMPA2	inositol(myo)-1(or 4)-monophosphatase 2
U79293	0.593793		Human clone 23948 mRNA sequence
NM_005557	-0.593746	KRT16	keratin 16 (focal non-epidermolytic palmoplantar keratoderma)
NM_002444	-0.592405	MSN	moesin
NM_003681	-0.592155	PDXK	pyridoxal (pyridoxine, vitamin B6) kinase
NM_006372	-0.591711	NSAP1	NS1-associated protein 1
NM_005218	-0.591192	DEFB1	defensin, beta 1
NM_004642	-0.591081	DOC1	deleted in oral cancer (mouse, homolog) 1
AL133074	0.590359		Homo sapiens cDNA: FLJ22139 fis, clone HEP20959
M73547	0.590317	D5S346	DNA segment, single copy probe LNS-CAI/LNS-CAII (deleted in polyposis
Contig65663	0.590312		ESTs
AL035297	-0.589728		H.sapiens gene from PAC 747L4
Contig35629_RC	0.589383		ESTs
NM_019027	0.588862	FLJ20273	hypothetical protein
NM_012425	-0.588804		Homo sapiens Ras suppressor protein 1 (RSU1), mRNA
NM_020179	-0.588326	FN5	FN5 protein
AF090913	-0.587275	TMSB10	thymosin, beta 10
NM_004176	0.587190	SREBF1	sterol regulatory element binding transcription factor 1
NM_016121	0.586941	LOC51133	NY-REN-45 antigen
NM_014773	0.586871	KIAA0141	KIAA0141 gene product
NM_019000	0.586677	FLJ20152	hypothetical protein
NM_016243	0.585942	LOC51706	cytochrome b5 reductase 1 (B5R.1)
NM_014274	-0.585815	ABP/ZF	Alu-binding protein with zinc finger domain
NM_018379	0.585497	FLJ11280	hypothetical protein FLJ11280
AL157431	-0.585077	DKFZp762A227	hypothetical protein DKFZp762A227
D38521	-0.584684	KIAA0077	KIAA0077 protein
NM_002570	0.584272	PACE4	paired basic amino acid cleaving system 4

Identifier	Correlation	Name	Description
NM_001809	-0.584252	CENPA	centromere protein A (17kD)
NM_003318	-0.583556	TTK	TTK protein kinase
NM_014325	-0.583555	CORO1C	coronin, actin-binding protein, 1C
NM_005667	0.583376	ZFP103	zinc finger protein homologous to Zfp103 in mouse
NM_004354	0.582420	CCNG2	cyclin G2
NM_003670	0.582235	BHLHB2	basic helix-loop-helix domain containing, class B, 2
NM_001673	-0.581902	ASNS	asparagine synthetase
NM_001333	-0.581402	CTSL2	cathepsin L2
Contig54295_RC	0.581256		ESTs
Contig33998_RC	0.581018		ESTs
NM_006002	-0.580592	UCHL3	ubiquitin carboxyl-terminal esterase L3 (ubiquitin thiolesterase)
NM_015392	0.580568	NPDC1	neural proliferation, differentiation and control, 1
NM_004866	0.580138	SCAMP1	secretory carrier membrane protein 1
Contig50391_RC	0.580071		ESTs
NM_000592	0.579965	C4B	complement component 4B
Contig50802_RC	0.579881		ESTs
Contig41635_RC	-0.579468		ESTs
NM_006845	-0.579339	KNSL6	kinesin-like 6 (mitotic centromere-associated kinesin)
NM_003720	-0.579296	DSCR2	Down syndrome critical region gene 2
NM_000060	0.578967	BTD	biotinidase
AL050388	-0.578736		Homo sapiens mRNA; cDNA DKFZp564M2422 (from clone DKFZp564M2422); partial cds
NM_003772	-0.578395	JRKL	jerky (mouse) homolog-like
NM_014398	-0.578388	TSC403	similar to lysosome-associated membrane glycoprotein
NM_001280	0.578213	CIRBP	cold inducible RNA-binding protein
NM_001395	-0.577369	DUSP9	dual specificity phosphatase 9
NM_016229	-0.576290	LOC51700	cytochrome b5 reductase b5R.2
NM_006096	-0.575615	NDRG1	N-myc downstream regulated
NM_001552	0.575438	IGFBP4	insulin-like growth factor-binding protein 4
NM_005558	-0.574818	LAD1	ladinin 1

Identifier	Correlation	Name	Description
Contig54534_RC	0.574784		Human glucose transporter pseudogene
Contig1239_RC	0.573822		Human Chromosome 16 BAC clone CIT987SK-A-362G6
Contig57173_RC	0.573807		Homo sapiens mRNA for KIAA1737 protein, partial cds
NM_004414	-0.573538	DSCR1	Down syndrome critical region gene 1
NM_021103	-0.572722	TMSB10	thymosin, beta 10
NM_002350	-0.571917	LYN	v-yes-1 Yamaguchi sarcoma viral related oncogene homolog
Contig51235_RC	0.571049		Homo sapiens cDNA: FLJ23388 fis, clone HEP17008
NM_013384	0.570987	TMSG1	tumor metastasis-suppressor
NM_014399	0.570936	NET-6	tetraspan NET-6 protein
Contig26022_RC	-0.570851		ESTs
AB023152	0.570561	KIAA0935	KIAA0935 protein
NM_021077	-0.569944	NMB	neuromedin B
NM_003498	-0.569129	SNN	stannin
U17077	-0.568979	BENE	BENE protein
D86985	0.567698	KIAA0232	KIAA0232 gene product
NM_006357	-0.567513	UBE2E3	ubiquitin-conjugating enzyme E2E 3 (homologous to yeast UBC4/5)
AL049397	-0.567434		Homo sapiens mRNA; cDNA DKFZp586C1019 (from clone DKFZp586C1019)
Contig64502	0.567433		ESTs, Weakly similar to unknown [M.musculus]
Contig56298_RC	-0.566892	FLJ13154	hypothetical protein FLJ13154
Contig46056_RC	0.566634		ESTs, Weakly similar to YZ28_HUMAN HYPOTHETICAL PROTEIN ZAP128 [H.sapiens]
AF007153	0.566044		Homo sapiens clone 23736 mRNA sequence
Contig1778_RC	-0.565789		ESTs
NM_017702	-0.565789	FLJ20186	hypothetical protein FLJ20186
Contig39226_RC	0.565761		Homo sapiens cDNA FLJ12187 fis, clone MAMMA1000831
NM_000168	0.564879	GLI3	GLI-Kruppel family member GLI3 (Greig cephalopolysyndactyly syndrome)

Identifier	Correlation	Name	Description
Contig57609_RC	0.564751		ESTs, Weakly similar to T2D3_HUMAN TRANSCRIPTION INITIATION FACTOR TFIID 135 KDA SUBUNIT [H.sapiens]
U45975	0.564602	PIB5PA	phosphatidylinositol (4,5) bisphosphate 5-phosphatase, A
AF038182	0.564596		Homo sapiens clone 23860 mRNA sequence
Contig5348_RC	0.564480		ESTs, Weakly similar to 1607338A transcription factor BTF3a [H.sapiens]
NM_001321	-0.564459	CSRP2	cysteine and glycine-rich protein 2
Contig25362_RC	-0.563801		ESTs
NM_001609	0.563782	ACADSB	acyl-Coenzyme A dehydrogenase, short/branched chain
Contig40146	0.563731		wi84e12.x1 NCI_CGAP_Kid12 Homo sapiens cDNA clone IMAGE:2400046 3' similar to SW:RASD_DICDI P03967 RAS-LIKE PROTEIN RASD ;, mRNA sequence.
NM_016002	0.563403	LOC51097	CGI-49 protein
Contig34303_RC	0.563157		Homo sapiens cDNA: FLJ21517 fis, clone COL05829
Contig55883_RC	0.563141		ESTs
NM_017961	0.562479	FLJ20813	hypothetical protein FLJ20813
M21551	-0.562340	NMB	neuromedin B
Contig3940_RC	-0.561956	YWHAH	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, eta polypeptide
AB033111	-0.561746	KIAA1285	KIAA1285 protein
Contig43410_RC	0.561678		ESTs
Contig42006_RC	-0.561677		ESTs
Contig57272_RC	0.561228		ESTs
G26403	-0.561068	YWHAH	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, eta polypeptide
NM_005915	-0.560813	MCM6	minichromosome maintenance deficient (mis5, S. pombe) 6
NM_003875	-0.560668	GMPS	guanine monphosphate synthetase
AK000142	0.559651	AK000142	Homo sapiens cDNA FLJ20135 fis, clone COL06818.

Identifier	Correlation	Name	Description
NM_002709	-0.559621	PPP1CB	protein phosphatase 1, catalytic subunit, beta isoform
NM_001276	-0.558868	CHI3L1	chitinase 3-like 1 (cartilage glycoprotein-39)
NM_002857	0.558862	PXF	peroxisomal farnesylated protein
Contig33815_RC	-0.558741	FLJ22833	hypothetical protein FLJ22833
NM_003740	-0.558491	KCNK5	potassium channel, subfamily K, member 5 (TASK-2)
Contig53646_RC	0.558455		ESTs
NM_005538	-0.558350	INHBC	inhibin, beta C
NM_002111	0.557860	HD	huntingtin (Huntington disease)
NM_003683	-0.557807	D21S2056E	DNA segment on chromosome 21 (unique) 2056 expressed sequence
NM_003035	-0.557380	SIL	TAL1 (SCL) interrupting locus
Contig4388_RC	-0.557216		Homo sapiens, Similar to integral membrane protein 3, clone MGC:3011, mRNA, complete cds
Contig38288_RC	-0.556426		ESTs, Weakly similar to ISHUSS protein disulfide-isomerase [H.sapiens]
NM_015417	0.556184	DKFZP434I114	DKFZP434I114 protein
NM_015507	-0.556138	EGFL6	EGF-like-domain, multiple 6
AF279865	0.555951	KIF13B	kinesin family member 13B
Contig31288_RC	-0.555754		ESTs
NM_002966	-0.555620	S100A10	S100 calcium-binding protein A10 (annexin II ligand, calpactin I, light polypeptide (p11))
NM_017585	-0.555476	SLC2A6	solute carrier family 2 (facilitated glucose transporter), member 6
NM_013296	-0.555367	HSU54999	LGN protein
NM_000224	0.554838	KRT18	keratin 18
Contig49270_RC	-0.554593	KIAA1553	KIAA1553 protein
NM_004848	-0.554538	ICB-1	basement membrane-induced gene
NM_007275	0.554278	FUS1	lung cancer candidate
NM_007044	-0.553550	KATNA1	katanin p60 (ATPase-containing) subunit A 1
Contig1829	0.553317		ESTs
AF272357	0.553286	NPDC1	neural proliferation, differentiation and control, 1

Identifier	Correlation	Name	Description
Contig57584_RC	-0.553080		Homo sapiens, Similar to gene rich cluster, C8 gene, clone MGC:2577, mRNA, complete cds
NM_003039	-0.552747	SLC2A5	solute carrier family 2 (facilitated glucose transporter), member 5
NM_014216	0.552321	ITPK1	inositol 1,3,4-triphosphate 5/6 kinase
NM_007027	-0.552064	TOPBP1	topoisomerase (DNA) II binding protein
AF118224	-0.551916	ST14	suppression of tumorigenicity 14 (colon carcinoma, matriptase, epithin)
X75315	-0.551853	HSRNASE B	seb4D
NM_012101	-0.551824	ATDC	ataxia-telangiectasia group D-associated protein
AL157482	-0.551329	FLJ23399	hypothetical protein FLJ23399
NM_012474	-0.551150	UMPK	uridine monophosphate kinase
Contig57081_RC	0.551103		ESTs
NM_006941	-0.551069	SOX10	SRY (sex determining region Y)-box 10
NM_004694	0.550932	SLC16A6	solute carrier family 16 (monocarboxylic acid transporters), member 6
Contig9541_RC	0.550680		ESTs
Contig20617_RC	0.550546		ESTs
NM_004252	0.550365	SLC9A3R 1	solute carrier family 9 (sodium/hydrogen exchanger), isoform 3 regulatory factor 1
NM_015641	-0.550200	DKFZP586 B2022	testin
NM_004336	-0.550164	BUB1	budding uninhibited by benzimidazoles 1 (yeast homolog)
Contig39960_RC	-0.549951	FLJ21079	hypothetical protein FLJ21079
NM_020686	0.549659	NPD009	NPD009 protein
NM_002633	-0.549647	PGM1	phosphoglucomutase 1
Contig30480_RC	0.548932		ESTs
NM_003479	0.548896	PTP4A2	protein tyrosine phosphatase type IVA, member 2
NM_001679	-0.548768	ATP1B3	ATPase, Na+/K+ transporting, beta 3 polypeptide
NM_001124	-0.548601	ADM	adrenomedullin
NM_001216	-0.548375	CA9	carbonic anhydrase IX

Identifier	Correlation	Name	Description
U58033	-0.548354	MTMR2	myotubularin related protein 2
NM_018389	-0.547875	FLJ11320	hypothetical protein FLJ11320
AF176012	0.547867	JDP1	J domain containing protein 1
Contig66705_RC	-0.546926	ST5	suppression of tumorigenicity 5
NM_018194	0.546878	FLJ10724	hypothetical protein FLJ10724
NM_006851	-0.546823	RTVP1	glioma pathogenesis-related protein
Contig53870_RC	0.546756		ESTs
NM_002482	-0.546012	NASP	nuclear autoantigenic sperm protein (histone-binding)
NM_002292	0.545949	LAMB2	laminin, beta 2 (laminin S)
NM_014696	-0.545758	KIAA0514	KIAA0514 gene product
Contig49855	0.545517		ESTs
AL117666	0.545203	DKFZP586O1624	DKFZP586O1624 protein
NM_004701	-0.545185	CCNB2	cyclin B2
NM_007050	0.544890	PTPRT	protein tyrosine phosphatase, receptor type, T
NM_000414	0.544778	HSD17B4	hydroxysteroid (17-beta) dehydrogenase 4
Contig52398_RC	-0.544775		Homo sapiens cDNA: FLJ21950 fis, clone HEP04949
AB007916	0.544496	KIAA0447	KIAA0447 gene product
Contig66219_RC	0.544467	FLJ22402	hypothetical protein FLJ22402
D87453	0.544145	KIAA0264	KIAA0264 protein
NM_015515	-0.543929	DKFZP434G032	DKFZP434G032 protein
NM_001530	-0.543898	HIF1A	hypoxia-inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)
NM_004109	-0.543893	FDX1	ferredoxin 1
NM_000381	-0.543871	MID1	midline 1 (Opitz/BBB syndrome)
Contig43983_RC	0.543523	CS2	calsyntenin-2
AL137761	0.543371		Homo sapiens mRNA; cDNA DKFZp586L2424 (from clone DKFZp586L2424)
NM_005764	-0.543175	DD96	epithelial protein up-regulated in carcinoma, membrane associated protein 17
Contig1838_RC	0.542996		Homo sapiens cDNA: FLJ22722 fis, clone HSI14444
NM_006670	0.542932	5T4	5T4 oncofetal trophoblast glycoprotein

Identifier	Correlation	Name	Description
Contig28552_RC	-0.542617		Homo sapiens mRNA; cDNA DKFZp434C0931 (from clone DKFZp434C0931); partial cds
Contig14284_RC	0.542224		ESTs
NM_006290	-0.542115	TNFAIP3	tumor necrosis factor, alpha-induced protein 3
AL050372	0.541463		Homo sapiens mRNA; cDNA DKFZp434A091 (from clone DKFZp434A091); partial cds
NM_014181	-0.541095	HSPC159	HSPC159 protein
Contig37141_RC	0.540990		Homo sapiens cDNA: FLJ23582 fis, clone LNG13759
NM_000947	-0.540621	PRIM2A	primase, polypeptide 2A (58kD)
NM_002136	0.540572	HNRPA1	heterogeneous nuclear ribonucleoprotein A1
NM_004494	-0.540543	HDGF	hepatoma-derived growth factor (high-mobility group protein 1-like)
Contig38983_RC	0.540526		ESTs
Contig27882_RC	-0.540506		ESTs
Z11887	-0.540020	MMP7	matrix metalloproteinase 7 (matrilysin, uterine)
NM_014575	-0.539725	SCHIP-1	schwannomin interacting protein 1
Contig38170_RC	0.539708		ESTs
Contig44064_RC	0.539403		ESTs
U68385	0.539395	MEIS3	Meis (mouse) homolog 3
Contig51967_RC	0.538952		ESTs
Contig37562_RC	0.538657		ESTs, Weakly similar to transformation-related protein [H.sapiens]
Contig40500_RC	0.538582		ESTs, Weakly similar to unnamed protein product [H.sapiens]
Contig1129_RC	0.538339		ESTs
NM_002184	0.538185	IL6ST	interleukin 6 signal transducer (gp130, oncostatin M receptor)
AL049381	0.538041		Homo sapiens cDNA FLJ12900 fis, clone NT2RP2004321
NM_002189	-0.537867	IL15RA	interleukin 15 receptor, alpha
NM_012110	-0.537562	CHIC2	cystein-rich hydrophobic domain 2
AB040881	-0.537473	KIAA1448	KIAA1448 protein
NM_016577	-0.537430	RAB6B	RAB6B, member RAS oncogene family
NM_001745	0.536940	CAMLG	calcium modulating ligand

Identifier	Correlation	Name	Description
NM_005742	-0.536738	P5	protein disulfide isomerase-related protein
AB011132	0.536345	KIAA0560	KIAA0560 gene product
Contig54898_RC	0.536094	PNN	pinin, desmosome associated protein
Contig45049_RC	-0.536043	FUT4	fucosyltransferase 4 (alpha (1,3) fucosyltransferase, myeloid-specific)
NM_006864	-0.535924	LILRB3	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 3
Contig53242_RC	-0.535909		Homo sapiens cDNA FLJ11436 fis, clone HEMBA1001213
NM_005544	0.535712	IRS1	insulin receptor substrate 1
Contig47456_RC	0.535493	CACNA1D	calcium channel, voltage-dependent, L type, alpha 1D subunit
Contig42751_RC	-0.535469		ESTs
Contig29126_RC	-0.535186		ESTs
NM_012391	0.535067	PDEF	prostate epithelium-specific Ets transcription factor
NM_012429	0.534974	SEC14L2	SEC14 (S. cerevisiae)-like 2
NM_018171	0.534898	FLJ10659	hypothetical protein FLJ10659
Contig53047_RC	-0.534773	TTYH1	tweety (Drosophila) homolog 1
Contig54968_RC	0.534754		Homo sapiens cDNA FLJ13558 fis, clone PLACE1007743
Contig2099_RC	-0.534694	KIAA1691	KIAA1691 protein
NM_005264	0.534057	GFRA1	GDNF family receptor alpha 1
NM_014036	-0.533638	SBBI42	BCM-like membrane protein precursor
NM_018101	-0.533473	FLJ10468	hypothetical protein FLJ10468
Contig56765_RC	0.533442		ESTs, Moderately similar to K02E10.2 [C.elegans]
AB006746	-0.533400	PLSCR1	phospholipid scramblase 1
NM_001089	0.533350	ABCA3	ATP-binding cassette, sub-family A (ABC1), member 3
NM_018188	-0.533132	FLJ10709	hypothetical protein FLJ10709
X94232	-0.532925	MAPRE2	microtubule-associated protein, RP/EB family, member 2
AF234532	-0.532910	MYO10	myosin X
Contig292_RC	0.532853	FLJ22386	hypothetical protein FLJ22386
NM_000101	-0.532767	CYBA	cytochrome b-245, alpha polypeptide
Contig47814_RC	-0.532656	HHGP	HHGP protein

Identifier	Correlation	Name	Description
NM_014320	-0.532430	SOUL	putative heme-binding protein
NM_020347	0.531976	LZTFL1	leucine zipper transcription factor-like 1
NM_004323	0.531936	BAG1	BCL2-associated athanogene
Contig50850_RC	-0.531914		ESTs
Contig11648_RC	0.531704		ESTs
NM_018131	-0.531559	FLJ10540	hypothetical protein FLJ10540
NM_004688	-0.531329	NMI	N-myc (and STAT) interactor
NM_014870	0.531101	KIAA0478	KIAA0478 gene product
Contig31424_RC	0.530720		ESTs
NM_000874	-0.530545	IFNAR2	interferon (alpha, beta and omega) receptor 2
Contig50588_RC	0.530145		ESTs
NM_016463	0.529998	HSPC195	hypothetical protein
NM_013324	0.529966	CISH	cytokine inducible SH2-containing protein
NM_006705	0.529840	GADD45G	growth arrest and DNA-damage-inducible, gamma
Contig38901_RC	-0.529747		ESTs
NM_004184	-0.529635	WARS	tryptophanyl-tRNA synthetase
NM_015955	-0.529538	LOC51072	CGI-27 protein
AF151810	0.529416	CGI-52	similar to phosphatidylcholine transfer protein 2
NM_002164	-0.529117	INDO	indoleamine-pyrrole 2,3 dioxygenase
NM_004267	-0.528679	CHST2	carbohydrate (chondroitin 6/keratan) sulfotransferase 2
Contig32185_RC	-0.528529		Homo sapiens cDNA FLJ13997 fis, clone Y79AA1002220
NM_004154	-0.528343	P2RY6	pyrimidinergic receptor P2Y, G-protein coupled, 6
NM_005235	0.528294	ERBB4	v-erb-a avian erythroblastic leukemia viral oncogene homolog-like 4
Contig40208_RC	-0.528062	LOC56938	transcription factor BMAL2
NM_013262	0.527297	MIR	myosin regulatory light chain interacting protein
NM_003034	-0.527148	SIAT8A	sialyltransferase 8 (alpha-N-acetylneuraminate: alpha-2,8-sialytransferase, GD3 synthase) A

Identifier	Correlation	Name	Description
NM_004556	-0.527146	NFKBIE	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, epsilon
NM_002046	-0.527051	GAPD	glyceraldehyde-3-phosphate dehydrogenase
NM_001905	-0.526986	CTPS	CTP synthase
Contig42402_RC	0.526852		ESTs
NM_014272	-0.526283	ADAMTS7	a disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 7
AF076612	0.526205	CHRD	chordin
Contig57725_RC	-0.526122		Homo sapiens mRNA for HMG-box transcription factor TCF-3, complete cds
Contig42041_RC	-0.525877		ESTs
Contig44656_RC	-0.525868		ESTs, Highly similar to S02392 alpha-2-macroglobulin receptor precursor [H.sapiens]
NM_018004	-0.525610	FLJ10134	hypothetical protein FLJ10134
Contig56434_RC	0.525510		Homo sapiens cDNA FLJ13603 fis, clone PLACE1010270
D25328	-0.525504	PFKP	phosphofructokinase, platelet
Contig55950_RC	-0.525358	FLJ22329	hypothetical protein FLJ22329
NM_002648	-0.525211	PIM1	pim-1 oncogene
AL157505	0.525186		Homo sapiens mRNA; cDNA DKFZp586P1124 (from clone DKFZp586P1124)
AF061034	-0.525185	FIP2	Homo sapiens FIP2 alternatively translated mRNA, complete cds.
NM_014721	-0.525102	KIAA0680	KIAA0680 gene product
NM_001634	-0.525030	AMD1	S-adenosylmethionine decarboxylase 1
NM_006304	-0.524911	DSS1	Deleted in split-hand/split-foot 1 region
Contig37778_RC	0.524667		ESTs, Highly similar to HLHUSB MHC class II histocompatibility antigen HLA-DP alpha-1 chain precursor [H.sapiens]
NM_003099	0.524339	SNX1	sorting nexin 1
AL079298	0.523774	MCCC2	methylcrotonoyl-Coenzyme A carboxylase 2 (beta)
NM_019013	-0.523663	FLJ10156	hypothetical protein

Identifier	Correlation	Name	Description
NM_000397	-0.523293	CYBB	cytochrome b-245, beta polypeptide (chronic granulomatous disease)
NM_014811	0.523132	KIAA0649	KIAA0649 gene product
Contig20600_RC	0.523072		ESTs
NM_005190	-0.522710	CCNC	cyclin C
AL161960	-0.522574	FLJ21324	hypothetical protein FLJ21324
AL117502	0.522280		Homo sapiens mRNA; cDNA DKFZp434D0935 (from clone DKFZp434D0935)
AF131753	-0.522245		Homo sapiens clone 24859 mRNA sequence
NM_000320	0.521974	QDPR	quinoid dihydropteridine reductase
NM_002115	-0.521870	HK3	hexokinase 3 (white cell)
NM_006460	0.521696	HIS1	HMBA-inducible
NM_018683	-0.521679	ZNF313	zinc finger protein 313
NM_004305	-0.521539	BIN1	bridging integrator 1
NM_006770	-0.521538	MARCO	macrophage receptor with collagenous structure
NM_001166	-0.521530	BIRC2	baculoviral IAP repeat-containing 2
D42047	0.521522	KIAA0089	KIAA0089 protein
NM_016235	-0.521298	GPRC5B	G protein-coupled receptor, family C, group 5, member B
NM_004504	-0.521189	HRB	HIV-1 Rev binding protein
NM_002727	-0.521146	PRG1	proteoglycan 1, secretory granule
AB029031	-0.520761	KIAA1108	KIAA1108 protein
NM_005556	-0.520692	KRT7	keratin 7
NM_018031	0.520600	WDR6	WD repeat domain 6
AL117523	-0.520579	KIAA1053	KIAA1053 protein
NM_004515	-0.520363	ILF2	interleukin enhancer binding factor 2, 45kD
NM_004708	-0.519935	PDCD5	programmed cell death 5
NM_005935	0.519765	MLLT2	myeloid/lymphoid or mixed-lineage leukemia (trithorax (Drosophila) homolog); translocated to, 2
Contig49289_RC	-0.519546		Homo sapiens mRNA; cDNA DKFZp586J1119 (from clone DKFZp586J1119); complete cds
NM_000211	-0.519342	ITGB2	integrin, beta 2 (antigen CD18 (p95), lymphocyte function-associated antigen 1; macrophage antigen 1 (mac-1) beta subunit)

Identifier	Correlation	Name	Description
AL079276	0.519207	LOC58495	putative zinc finger protein from EUROIMAGE 566589
Contig57825_RC	0.519041		ESTs
NM_002466	-0.518911	MYBL2	v-myb avian myeloblastosis viral oncogene homolog-like 2
NM_016072	-0.518802	LOC51026	CGI-141 protein
AB007950	-0.518699	KIAA0481	KIAA0481 gene product
NM_001550	-0.518549	IFRD1	interferon-related developmental regulator 1
AF155120	-0.518221	UBE2V1	ubiquitin-conjugating enzyme E2 variant 1
Contig49849_RC	0.517983		ESTs, Weakly similar to AF188706 1 g20 protein [H.sapiens]
NM_016625	-0.517936	LOC51319	hypothetical protein
NM_004049	-0.517862	BCL2A1	BCL2-related protein A1
Contig50719_RC	0.517740		ESTs
D80010	-0.517620	LPIN1	lipin 1
NM_000299	-0.517405	PKP1	plakophilin 1 (ectodermal dysplasia/skin fragility syndrome)
AL049365	0.517080	FTL	ferritin, light polypeptide
Contig65227	0.517003		ESTs
NM_004865	-0.516808	TBPL1	TBP-like 1
Contig54813_RC	0.516246	FLJ13962	hypothetical protein FLJ13962
NM_003494	-0.516221	DYSF	dysferlin, limb girdle muscular dystrophy 2B (autosomal recessive)
NM_004431	-0.516212	EPHA2	EphA2
AL117600	-0.516067	DKFZP564J0863	DKFZP564J0863 protein
AL080209	-0.516037	DKFZP586F2423	hypothetical protein DKFZp586F2423
NM_000135	-0.515613	FANCA	Fanconi anemia, complementation group A
NM_000050	-0.515494	ASS	argininosuccinate synthetase
NM_001830	-0.515439	CLCN4	chloride channel 4
NM_018234	-0.515365	FLJ10829	hypothetical protein FLJ10829
Contig53307_RC	0.515328		ESTs, Highly similar to KIAA1437 protein [H.sapiens]
AL117617	-0.515141		Homo sapiens mRNA; cDNA DKFZp564H0764 (from clone DKFZp564H0764)
NM_002906	-0.515098	RDX	radixin

Identifier	Correlation	Name	Description
NM_003360	-0.514427	UGT8	UDP glycosyltransferase 8 (UDP-galactose ceramide galactosyltransferase)
NM_018478	0.514332	HSMNP1	uncharacterized hypothalamus protein HSMNP1
M90657	-0.513908	TM4SF1	transmembrane 4 superfamily member 1
NM_014967	0.513793	KIAA1018	KIAA1018 protein
Contig1462_RC	0.513604	C11ORF15	chromosome 11 open reading frame 15
Contig37287_RC	-0.513324		ESTs
NM_000355	-0.513225	TCN2	transcobalamin II; macrocytic anemia
AB037756	0.512914	KIAA1335	hypothetical protein KIAA1335
Contig842_RC	-0.512880		ESTs
NM_018186	-0.512878	FLJ10706	hypothetical protein FLJ10706
NM_014668	0.512746	KIAA0575	KIAA0575 gene product
NM_003226	0.512611	TFF3	trefoil factor 3 (intestinal)
Contig56457_RC	-0.512548	TMEFF1	transmembrane protein with EGF-like and two follistatin-like domains 1
AL050367	-0.511999		Homo sapiens mRNA; cDNA DKFZp564A026 (from clone DKFZp564A026)
NM_014791	-0.511963	KIAA0175	KIAA0175 gene product
Contig36312_RC	0.511794		ESTs
NM_004811	-0.511447	LPXN	leupaxin
Contig67182_RC	-0.511416		ESTs, Highly similar to epithelial V-like antigen precursor [H.sapiens]
Contig52723_RC	-0.511134		ESTs
Contig17105_RC	-0.511072		Homo sapiens mRNA for putative cytoplasmatic protein (ORF1-FL21)
NM_014449	0.511023	A	protein "A"
Contig52957_RC	0.510815		ESTs
Contig49388_RC	0.510582	FLJ13322	hypothetical protein FLJ13322
NM_017786	0.510557	FLJ20366	hypothetical protein FLJ20366
AL157476	0.510478		Homo sapiens mRNA; cDNA DKFZp761C082 (from clone DKFZp761C082)
NM_001919	0.510242	DCI	dodecenoyl-Coenzyme A delta isomerase (3,2 trans-enoyl-Coenzyme A isomerase)

Identifier	Correlation	Name	Description
NM_000268	-0.510165	NF2	neurofibromin 2 (bilateral acoustic neuroma)
NM_016210	0.510018	LOC51161	g20 protein
Contig45816_RC	-0.509977		ESTs
NM_003953	-0.509969	MPZL1	myelin protein zero-like 1
NM_000057	-0.509669	BLM	Bloom syndrome
NM_014452	-0.509473	DR6	death receptor 6
Contig45156_RC	0.509284		ESTs, Moderately similar to motor domain of KIF12 [M.musculus]
NM_006943	0.509149	SOX22	SRY (sex determining region Y)-box 22
NM_000594	-0.509012	TNF	tumor necrosis factor (TNF superfamily, member 2)
AL137316	-0.508353	KIAA1609	KIAA1609 protein
NM_000557	-0.508325	GDF5	growth differentiation factor 5 (cartilage-derived morphogenetic protein-1)
NM_018685	-0.508307	ANLN	anillin (Drosophila Scraps homolog), actin binding protein
Contig53401_RC	0.508189		ESTs
NM_014364	-0.508170	GAPDS	glyceraldehyde-3-phosphate dehydrogenase, testis-specific
Contig50297_RC	0.508137		ESTs, Moderately similar to ALU8_HUMAN ALU SUBFAMILY SX SEQUENCE CONTAMINATION WARNING ENTRY [H.sapiens]
Contig51800	0.507891		ESTs, Weakly similar to ALU6_HUMAN ALU SUBFAMILY SP SEQUENCE CONTAMINATION WARNING ENTRY [H.sapiens]
Contig49098_RC	-0.507716	MGC4090	hypothetical protein MGC4090
NM_002985	-0.507554	SCYA5	small inducible cytokine A5 (RANTES)
AB007899	0.507439	KIAA0439	KIAA0439 protein; homolog of yeast ubiquitin-protein ligase Rsp5
AL110139	0.507145		Homo sapiens mRNA; cDNA DKFZp564O1763 (from clone DKFZp564O1763)
Contig51117_RC	0.507001		ESTs
NM_017660	-0.506768	FLJ20085	hypothetical protein FLJ20085
NM_018000	0.506686	FLJ10116	hypothetical protein FLJ10116
NM_005555	-0.506516	KRT6B	keratin 6B

Identifier	Correlation	Name	Description
NM_005582	-0.506462	LY64	lymphocyte antigen 64 (mouse) homolog, radioprotective, 105kD
Contig47405_RC	0.506202		ESTs
NM_014808	0.506173	KIAA0793	KIAA0793 gene product
NM_004938	-0.506121	DAPK1	death-associated protein kinase 1
NM_020659	-0.505793	TTYH1	tweety (Drosophila) homolog 1
NM_006227	-0.505604	PLTP	phospholipid transfer protein
NM_014268	-0.505412	MAPRE2	microtubule-associated protein, RP/EB family, member 2
NM_004711	0.504849	SYNGR1	synaptogyrin 1
NM_004418	-0.504497	DUSP2	dual specificity phosphatase 2
NM_003508	-0.504475	FZD9	frizzled (Drosophila) homolog 9

Table 3. 430 gene markers that distinguish *BRCA1*-related tumor samples from sporadic tumor samples

GenBank Accession Number	SEQ ID NO	GenBank Accession Number	SEQ ID NO
AB002301	SEQ ID NO 4	NM_012391	SEQ ID NO 1406
AB004857	SEQ ID NO 8	NM_012428	SEQ ID NO 1412
AB007458	SEQ ID NO 12	NM_013233	SEQ ID NO 1418
AB014534	SEQ ID NO 29	NM_013253	SEQ ID NO 1422
AB018305	SEQ ID NO 34	NM_013262	SEQ ID NO 1425
AB020677	SEQ ID NO 36	NM_013372	SEQ ID NO 1434
AB020689	SEQ ID NO 37	NM_013378	SEQ ID NO 1435
AB023151	SEQ ID NO 41	NM_014096	SEQ ID NO 1450
AB023163	SEQ ID NO 43	NM_014242	SEQ ID NO 1464
AB028986	SEQ ID NO 48	NM_014314	SEQ ID NO 1472
AB029025	SEQ ID NO 50	NM_014398	SEQ ID NO 1486
AB032966	SEQ ID NO 53	NM_014402	SEQ ID NO 1488
AB032988	SEQ ID NO 57	NM_014476	SEQ ID NO 1496
AB033049	SEQ ID NO 63	NM_014521	SEQ ID NO 1499
AB033055	SEQ ID NO 66	NM_014585	SEQ ID NO 1504
AB037742	SEQ ID NO 73	NM_014597	SEQ ID NO 1506
AB041269	SEQ ID NO 96	NM_014642	SEQ ID NO 1510
AF000974	SEQ ID NO 97	NM_014679	SEQ ID NO 1517
AF042838	SEQ ID NO 111	NM_014680	SEQ ID NO 1518
AF052155	SEQ ID NO 119	NM_014700	SEQ ID NO 1520
AF055084	SEQ ID NO 125	NM_014723	SEQ ID NO 1523
AF063725	SEQ ID NO 129	NM_014770	SEQ ID NO 1530
AF070536	SEQ ID NO 133	NM_014785	SEQ ID NO 1534
AF070617	SEQ ID NO 135	NM_014817	SEQ ID NO 1539
AF073299	SEQ ID NO 136	NM_014840	SEQ ID NO 1541
AF079529	SEQ ID NO 140	NM_014878	SEQ ID NO 1546
AF090353	SEQ ID NO 141	NM_015493	SEQ ID NO 1564
AF116238	SEQ ID NO 155	NM_015523	SEQ ID NO 1568
AF151810	SEQ ID NO 171	NM_015544	SEQ ID NO 1570
AF220492	SEQ ID NO 185	NM_015623	SEQ ID NO 1572
AJ224741	SEQ ID NO 196	NM_015640	SEQ ID NO 1573
AJ250475	SEQ ID NO 201	NM_015721	SEQ ID NO 1576
AJ270996	SEQ ID NO 202	NM_015881	SEQ ID NO 1577
AJ272057	SEQ ID NO 203	NM_015937	SEQ ID NO 1582
AK000174	SEQ ID NO 211	NM_015964	SEQ ID NO 1586

GenBank Accession Number	SEQ ID NO	GenBank Accession Number	SEQ ID NO
AK000617	SEQ ID NO 215	NM_015984	SEQ ID NO 1587
AK000959	SEQ ID NO 222	NM_016000	SEQ ID NO 1591
AK001438	SEQ ID NO 229	NM_016018	SEQ ID NO 1593
AK001838	SEQ ID NO 233	NM_016066	SEQ ID NO 1601
AK002107	SEQ ID NO 238	NM_016073	SEQ ID NO 1603
AK002197	SEQ ID NO 239	NM_016081	SEQ ID NO 1604
AL035297	SEQ ID NO 241	NM_016140	SEQ ID NO 1611
AL049346	SEQ ID NO 243	NM_016223	SEQ ID NO 1622
AL049370	SEQ ID NO 245	NM_016267	SEQ ID NO 1629
AL049667	SEQ ID NO 249	NM_016307	SEQ ID NO 1633
AL080222	SEQ ID NO 276	NM_016364	SEQ ID NO 1639
AL096737	SEQ ID NO 279	NM_016373	SEQ ID NO 1640
AL110163	SEQ ID NO 282	NM_016459	SEQ ID NO 1646
AL133057	SEQ ID NO 300	NM_016471	SEQ ID NO 1648
AL133096	SEQ ID NO 302	NM_016548	SEQ ID NO 1654
AL133572	SEQ ID NO 305	NM_016620	SEQ ID NO 1662
AL133619	SEQ ID NO 307	NM_016820	SEQ ID NO 1674
AL133623	SEQ ID NO 309	NM_017423	SEQ ID NO 1678
AL137347	SEQ ID NO 320	NM_017709	SEQ ID NO 1698
AL137381	SEQ ID NO 322	NM_017732	SEQ ID NO 1700
AL137461	SEQ ID NO 325	NM_017734	SEQ ID NO 1702
AL137540	SEQ ID NO 328	NM_017750	SEQ ID NO 1704
AL137555	SEQ ID NO 329	NM_017763	SEQ ID NO 1706
AL137638	SEQ ID NO 332	NM_017782	SEQ ID NO 1710
AL137639	SEQ ID NO 333	NM_017816	SEQ ID NO 1714
AL137663	SEQ ID NO 334	NM_018043	SEQ ID NO 1730
AL137761	SEQ ID NO 339	NM_018072	SEQ ID NO 1734
AL157431	SEQ ID NO 340	NM_018093	SEQ ID NO 1738
AL161960	SEQ ID NO 351	NM_018103	SEQ ID NO 1742
AL355708	SEQ ID NO 353	NM_018171	SEQ ID NO 1751
AL359053	SEQ ID NO 354	NM_018187	SEQ ID NO 1755
D26488	SEQ ID NO 359	NM_018188	SEQ ID NO 1756
D38521	SEQ ID NO 361	NM_018222	SEQ ID NO 1761
D50914	SEQ ID NO 367	NM_018228	SEQ ID NO 1762
D80001	SEQ ID NO 369	NM_018373	SEQ ID NO 1777
G26403	SEQ ID NO 380	NM_018390	SEQ ID NO 1781
K02276	SEQ ID NO 383	NM_018422	SEQ ID NO 1784

GenBank Accession Number	SEQ ID NO	GenBank Accession Number	SEQ ID NO
M21551	SEQ ID NO 394	NM_018509	SEQ ID NO 1792
M27749	SEQ ID NO 397	NM_018584	SEQ ID NO 1796
M28170	SEQ ID NO 398	NM_018653	SEQ ID NO 1797
M73547	SEQ ID NO 409	NM_018660	SEQ ID NO 1798
M80899	SEQ ID NO 411	NM_018683	SEQ ID NO 1799
NM_000067	SEQ ID NO 423	NM_019049	SEQ ID NO 1814
NM_000087	SEQ ID NO 427	NM_019063	SEQ ID NO 1815
NM_000090	SEQ ID NO 428	NM_020150	SEQ ID NO 1823
NM_000165	SEQ ID NO 444	NM_020987	SEQ ID NO 1848
NM_000168	SEQ ID NO 445	NM_021095	SEQ ID NO 1855
NM_000196	SEQ ID NO 449	NM_021242	SEQ ID NO 1867
NM_000269	SEQ ID NO 457	U41387	SEQ ID NO 1877
NM_000310	SEQ ID NO 466	U45975	SEQ ID NO 1878
NM_000396	SEQ ID NO 479	U58033	SEQ ID NO 1881
NM_000397	SEQ ID NO 480	U67784	SEQ ID NO 1884
NM_000597	SEQ ID NO 502	U68385	SEQ ID NO 1885
NM_000636	SEQ ID NO 509	U80736	SEQ ID NO 1890
NM_000888	SEQ ID NO 535	X00437	SEQ ID NO 1899
NM_000903	SEQ ID NO 536	X07203	SEQ ID NO 1904
NM_000930	SEQ ID NO 540	X16302	SEQ ID NO 1907
NM_000931	SEQ ID NO 541	X51630	SEQ ID NO 1908
NM_000969	SEQ ID NO 547	X57809	SEQ ID NO 1912
NM_000984	SEQ ID NO 548	X57819	SEQ ID NO 1913
NM_001026	SEQ ID NO 552	X58529	SEQ ID NO 1914
NM_001054	SEQ ID NO 554	X66087	SEQ ID NO 1916
NM_001179	SEQ ID NO 567	X69150	SEQ ID NO 1917
NM_001184	SEQ ID NO 568	X72475	SEQ ID NO 1918
NM_001204	SEQ ID NO 571	X74794	SEQ ID NO 1920
NM_001206	SEQ ID NO 572	X75315	SEQ ID NO 1921
NM_001218	SEQ ID NO 575	X84340	SEQ ID NO 1925
NM_001275	SEQ ID NO 586	X98260	SEQ ID NO 1928
NM_001394	SEQ ID NO 602	Y07512	SEQ ID NO 1931
NM_001424	SEQ ID NO 605	Y14737	SEQ ID NO 1932
NM_001448	SEQ ID NO 610	Z34893	SEQ ID NO 1934
NM_001504	SEQ ID NO 620	Contig237_RC	SEQ ID NO 1940
NM_001553	SEQ ID NO 630	Contig292_RC	SEQ ID NO 1942
NM_001674	SEQ ID NO 646	Contig372_RC	SEQ ID NO 1943

GenBank Accession Number	SEQ ID NO	GenBank Accession Number	SEQ ID NO
NM_001675	SEQ ID NO 647	Contig756_RC	SEQ ID NO 1955
NM_001725	SEQ ID NO 652	Contig842_RC	SEQ ID NO 1958
NM_001740	SEQ ID NO 656	Contig1632_RC	SEQ ID NO 1977
NM_001756	SEQ ID NO 659	Contig1826_RC	SEQ ID NO 1980
NM_001770	SEQ ID NO 664	Contig2237_RC	SEQ ID NO 1988
NM_001797	SEQ ID NO 670	Contig2915_RC	SEQ ID NO 2003
NM_001845	SEQ ID NO 680	Contig3164_RC	SEQ ID NO 2007
NM_001873	SEQ ID NO 684	Contig3252_RC	SEQ ID NO 2008
NM_001888	SEQ ID NO 687	Contig3940_RC	SEQ ID NO 2018
NM_001892	SEQ ID NO 688	Contig9259_RC	SEQ ID NO 2039
NM_001919	SEQ ID NO 694	Contig10268_RC	SEQ ID NO 2041
NM_001946	SEQ ID NO 698	Contig10437_RC	SEQ ID NO 2043
NM_001953	SEQ ID NO 699	Contig10973_RC	SEQ ID NO 2044
NM_001960	SEQ ID NO 704	Contig14390_RC	SEQ ID NO 2054
NM_001985	SEQ ID NO 709	Contig16453_RC	SEQ ID NO 2060
NM_002023	SEQ ID NO 712	Contig16759_RC	SEQ ID NO 2061
NM_002051	SEQ ID NO 716	Contig19551	SEQ ID NO 2070
NM_002053	SEQ ID NO 717	Contig24541_RC	SEQ ID NO 2088
NM_002164	SEQ ID NO 734	Contig25362_RC	SEQ ID NO 2093
NM_002200	SEQ ID NO 739	Contig25617_RC	SEQ ID NO 2094
NM_002201	SEQ ID NO 740	Contig25722_RC	SEQ ID NO 2096
NM_002213	SEQ ID NO 741	Contig26022_RC	SEQ ID NO 2099
NM_002250	SEQ ID NO 747	Contig27915_RC	SEQ ID NO 2114
NM_002512	SEQ ID NO 780	Contig28081_RC	SEQ ID NO 2116
NM_002542	SEQ ID NO 784	Contig28179_RC	SEQ ID NO 2118
NM_002561	SEQ ID NO 786	Contig28550_RC	SEQ ID NO 2119
NM_002615	SEQ ID NO 793	Contig29639_RC	SEQ ID NO 2127
NM_002686	SEQ ID NO 803	Contig29647_RC	SEQ ID NO 2128
NM_002709	SEQ ID NO 806	Contig30092_RC	SEQ ID NO 2130
NM_002742	SEQ ID NO 812	Contig30209_RC	SEQ ID NO 2132
NM_002775	SEQ ID NO 815	Contig32185_RC	SEQ ID NO 2156
NM_002975	SEQ ID NO 848	Contig32798_RC	SEQ ID NO 2161
NM_002982	SEQ ID NO 849	Contig33230_RC	SEQ ID NO 2163
NM_003104	SEQ ID NO 870	Contig33394_RC	SEQ ID NO 2165
NM_003118	SEQ ID NO 872	Contig36323_RC	SEQ ID NO 2197
NM_003144	SEQ ID NO 876	Contig36761_RC	SEQ ID NO 2201
NM_003165	SEQ ID NO 882	Contig37141_RC	SEQ ID NO 2209

GenBank Accession Number	SEQ ID NO	GenBank Accession Number	SEQ ID NO
NM_003197	SEQ ID NO 885	Contig37778_RC	SEQ ID NO 2218
NM_003202	SEQ ID NO 886	Contig38285_RC	SEQ ID NO 2222
NM_003217	SEQ ID NO 888	Contig38520_RC	SEQ ID NO 2225
NM_003283	SEQ ID NO 898	Contig38901_RC	SEQ ID NO 2232
NM_003462	SEQ ID NO 911	Contig39826_RC	SEQ ID NO 2241
NM_003500	SEQ ID NO 918	Contig40212_RC	SEQ ID NO 2251
NM_003561	SEQ ID NO 925	Contig40712_RC	SEQ ID NO 2257
NM_003607	SEQ ID NO 930	Contig41402_RC	SEQ ID NO 2265
NM_003633	SEQ ID NO 933	Contig41635_RC	SEQ ID NO 2272
NM_003641	SEQ ID NO 934	Contig42006_RC	SEQ ID NO 2280
NM_003683	SEQ ID NO 943	Contig42220_RC	SEQ ID NO 2286
NM_003729	SEQ ID NO 949	Contig42306_RC	SEQ ID NO 2287
NM_003793	SEQ ID NO 954	Contig43918_RC	SEQ ID NO 2312
NM_003829	SEQ ID NO 958	Contig44195_RC	SEQ ID NO 2316
NM_003866	SEQ ID NO 961	Contig44265_RC	SEQ ID NO 2318
NM_003904	SEQ ID NO 967	Contig44278_RC	SEQ ID NO 2319
NM_003953	SEQ ID NO 974	Contig44757_RC	SEQ ID NO 2329
NM_004024	SEQ ID NO 982	Contig45588_RC	SEQ ID NO 2349
NM_004053	SEQ ID NO 986	Contig46262_RC	SEQ ID NO 2361
NM_004295	SEQ ID NO 1014	Contig46288_RC	SEQ ID NO 2362
NM_004438	SEQ ID NO 1038	Contig46343_RC	SEQ ID NO 2363
NM_004559	SEQ ID NO 1057	Contig46452_RC	SEQ ID NO 2366
NM_004616	SEQ ID NO 1065	Contig46868_RC	SEQ ID NO 2373
NM_004741	SEQ ID NO 1080	Contig46937_RC	SEQ ID NO 2377
NM_004772	SEQ ID NO 1084	Contig48004_RC	SEQ ID NO 2393
NM_004791	SEQ ID NO 1086	Contig48249_RC	SEQ ID NO 2397
NM_004848	SEQ ID NO 1094	Contig48774_RC	SEQ ID NO 2405
NM_004866	SEQ ID NO 1097	Contig48913_RC	SEQ ID NO 2411
NM_005128	SEQ ID NO 1121	Contig48945_RC	SEQ ID NO 2412
NM_005148	SEQ ID NO 1124	Contig48970_RC	SEQ ID NO 2413
NM_005196	SEQ ID NO 1127	Contig49233_RC	SEQ ID NO 2419
NM_005326	SEQ ID NO 1140	Contig49289_RC	SEQ ID NO 2422
NM_005518	SEQ ID NO 1161	Contig49342_RC	SEQ ID NO 2423
NM_005538	SEQ ID NO 1163	Contig49510_RC	SEQ ID NO 2430
NM_005557	SEQ ID NO 1170	Contig49855	SEQ ID NO 2440
NM_005718	SEQ ID NO 1189	Contig49948_RC	SEQ ID NO 2442
NM_005804	SEQ ID NO 1201	Contig50297_RC	SEQ ID NO 2451

GenBank Accession Number	SEQ ID NO	GenBank Accession Number	SEQ ID NO
NM_005824	SEQ ID NO 1203	Contig50669_RC	SEQ ID NO 2458
NM_005935	SEQ ID NO 1220	Contig50673_RC	SEQ ID NO 2459
NM_006002	SEQ ID NO 1225	Contig50838_RC	SEQ ID NO 2465
NM_006148	SEQ ID NO 1249	Contig51068_RC	SEQ ID NO 2471
NM_006235	SEQ ID NO 1257	Contig51929	SEQ ID NO 2492
NM_006271	SEQ ID NO 1261	Contig51953_RC	SEQ ID NO 2493
NM_006287	SEQ ID NO 1264	Contig52405_RC	SEQ ID NO 2502
NM_006296	SEQ ID NO 1267	Contig52543_RC	SEQ ID NO 2505
NM_006378	SEQ ID NO 1275	Contig52720_RC	SEQ ID NO 2513
NM_006461	SEQ ID NO 1287	Contig53281_RC	SEQ ID NO 2530
NM_006573	SEQ ID NO 1300	Contig53598_RC	SEQ ID NO 2537
NM_006622	SEQ ID NO 1302	Contig53757_RC	SEQ ID NO 2543
NM_006696	SEQ ID NO 1308	Contig53944_RC	SEQ ID NO 2545
NM_006769	SEQ ID NO 1316	Contig54425	SEQ ID NO 2561
NM_006787	SEQ ID NO 1319	Contig54547_RC	SEQ ID NO 2565
NM_006875	SEQ ID NO 1334	Contig54757_RC	SEQ ID NO 2574
NM_006885	SEQ ID NO 1335	Contig54916_RC	SEQ ID NO 2581
NM_006918	SEQ ID NO 1339	Contig55770_RC	SEQ ID NO 2604
NM_006923	SEQ ID NO 1340	Contig55801_RC	SEQ ID NO 2606
NM_006941	SEQ ID NO 1342	Contig56143_RC	SEQ ID NO 2619
NM_007070	SEQ ID NO 1354	Contig56160_RC	SEQ ID NO 2620
NM_007088	SEQ ID NO 1356	Contig56303_RC	SEQ ID NO 2626
NM_007146	SEQ ID NO 1358	Contig57023_RC	SEQ ID NO 2639
NM_007173	SEQ ID NO 1359	Contig57138_RC	SEQ ID NO 2644
NM_007246	SEQ ID NO 1366	Contig57609_RC	SEQ ID NO 2657
NM_007358	SEQ ID NO 1374	Contig58301_RC	SEQ ID NO 2667
NM_012135	SEQ ID NO 1385	Contig58512_RC	SEQ ID NO 2670
NM_012151	SEQ ID NO 1387	Contig60393	SEQ ID NO 2674
NM_012258	SEQ ID NO 1396	Contig60509_RC	SEQ ID NO 2675
NM_012317	SEQ ID NO 1399	Contig61254_RC	SEQ ID NO 2677
NM_012337	SEQ ID NO 1403	Contig62306	SEQ ID NO 2680
NM_012339	SEQ ID NO 1404	Contig64502	SEQ ID NO 2689

Table 4. 100 preferred markers from Table 3 distinguishing *BRCA1*-related tumors from sporadic tumors.

Identifier	Correlation	Sequence Name	Description
NM_001892	-0.651689	CSNK1A1	casein kinase 1, alpha 1
NM_018171	-0.637696	FLJ10659	hypothetical protein FLJ10659
Contig40712_RC	-0.612509		ESTs
NM_001204	-0.608470	BMPR2	bone morphogenetic protein receptor, type II (serine/threonine kinase)
NM_005148	-0.598612	UNC119	unc119 (C.elegans) homolog
G26403	0.585054	YWHAH	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, eta polypeptide
NM_015640	0.583397	PAI-RBP1	PAI-1 mRNA-binding protein
Contig9259_RC	0.581362		ESTs
AB033049	-0.578750	KIAA1223	KIAA1223 protein
NM_015523	0.576029	DKFZP566E144	small fragment nuclease
Contig41402_RC	-0.571650		Human DNA sequence from clone RP11-16L21 on chromosome 9. Contains the gene for NADP-dependent leukotriene B4 12-hydroxydehydrogenase, the gene for a novel DnaJ domain protein similar to Drosophila, C. elegans and Arabidopsis predicted proteins, the GNG10 gene for guanine nucleotide binding protein 10, a novel gene, ESTs, STSs, GSSs and six CpG islands
NM_004791	-0.564819	ITGBL1	integrin, beta-like 1 (with EGF-like repeat domains)
NM_007070	0.561173	FAP48	FKBP-associated protein
NM_014597	0.555907	HSU15552	acidic 82 kDa protein mRNA
AF000974	0.547194	TRIP6	thyroid hormone receptor interactor 6
NM_016073	-0.547072	CGI-142	CGI-142
Contig3940_RC	0.544073	YWHAH	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, eta polypeptide
NM_003683	0.542219	D21S2056E	DNA segment on chromosome 21 (unique) 2056 expressed sequence

Identifier	Correlation	Sequence Name	Description
Contig58512_RC	-0.528458		Homo sapiens pancreas tumor-related protein (FKSG12) mRNA, complete cds
NM_003904	0.521223	ZNF259	zinc finger protein 259
Contig26022_RC	0.517351		ESTs
Contig48970_RC	-0.516953	KIAA0892	KIAA0892 protein
NM_016307	-0.515398	PRX2	paired related homeobox protein
AL137761	-0.514891		Homo sapiens mRNA; cDNA DKFZp586L2424 (from clone DKFZp586L2424)
NM_001919	-0.514799	DCI	dodecenoyl-Coenzyme A delta isomerase (3,2 trans-enoyl-Coenzyme A isomerase)
NM_000196	-0.514004	HSD11B2	hydroxysteroid (11-beta) dehydrogenase 2
NM_002200	0.513149	IRF5	interferon regulatory factor 5
AL133572	0.511340		Homo sapiens mRNA; cDNA DKFZp434I0535 (from clone DKFZp434I0535); partial cds
NM_019063	0.511127	C2ORF2	chromosome 2 open reading frame 2
Contig25617_RC	0.509506		ESTs
NM_007358	0.508145	M96	putative DNA binding protein
NM_014785	-0.507114	KIAA0258	KIAA0258 gene product
NM_006235	0.506585	POU2AF1	POU domain, class 2, associating factor 1
NM_014680	-0.505779	KIAA0100	KIAA0100 gene product
X66087	0.500842	MYBL1	v-myb avian myeloblastosis viral oncogene homolog-like 1
Y07512	-0.500686	PRKG1	protein kinase, cGMP-dependent, type I
NM_006296	0.500344	VRK2	vaccinia related kinase 2
Contig44278_RC	0.498260	DKFZP434K114	DKFZP434K114 protein
Contig56160_RC	-0.497695		ESTs
NM_002023	-0.497570	FMOD	fibromodulin
M28170	0.497095	CD19	CD19 antigen
D26488	0.496511	KIAA0007	KIAA0007 protein
X72475	0.496125		H.sapiens mRNA for rearranged Ig kappa light chain variable region (l.114)

Identifier	Correlation	Sequence Name	Description
K02276	0.496068	MYC	v-myc avian myelocytomatosis viral oncogene homolog
NM_013378	0.495648	VPREB3	pre-B lymphocyte gene 3
X58529	0.495608	IGHM	immunoglobulin heavy constant mu
NM_000168	-0.494260	GLI3	GLI-Kruppel family member GLI3 (Greig cephalopolysyndactyly syndrome)
NM_004866	-0.492967	SCAMP1	secretory carrier membrane protein 1
NM_013253	-0.491159	DKK3	dickkopf (Xenopus laevis) homolog 3
NM_003729	0.488971	RPC	RNA 3'-terminal phosphate cyclase
NM_006875	0.487407	PIM2	pim-2 oncogene
NM_018188	0.487126	FLJ10709	hypothetical protein FLJ10709
NM_004848	0.485408	ICB-1	basement membrane-induced gene
NM_001179	0.483253	ART3	ADP-ribosyltransferase 3
NM_016548	-0.482329	LOC51280	golgi membrane protein GP73
NM_007146	-0.481994	ZNF161	zinc finger protein 161
NM_021242	-0.481754	STRAIT11499	hypothetical protein STRAIT11499
NM_016223	0.481710	PACSIN3	protein kinase C and casein kinase substrate in neurons 3
NM_003197	-0.481526	TCEB1L	transcription elongation factor B (SIII), polypeptide 1-like
NM_000067	-0.481003	CA2	carbonic anhydrase II
NM_006885	-0.479705	ATBF1	AT-binding transcription factor 1
NM_002542	0.478282	OGG1	8-oxoguanine DNA glycosylase
AL133619	-0.476596		Homo sapiens mRNA; cDNA DKFZp434E2321 (from clone DKFZp434E2321); partial cds
D80001	0.476130	KIAA0179	KIAA0179 protein
NM_018660	-0.475548	LOC55893	papillomavirus regulatory factor PRF-1
AB004857	0.473440	SLC11A2	solute carrier family 11 (proton-coupled divalent metal ion transporters), member 2
NM_002250	0.472900	KCNN4	potassium intermediate/small conductance calcium-activated channel, subfamily N, member 4
Contig56143_RC	-0.472611		ESTs, Weakly similar to A54849 collagen alpha 1(VII) chain precursor [H.sapiens]

Identifier	Correlation	Sequence Name	Description
NM_001960	0.471502	EEF1D	eukaryotic translation elongation factor 1 delta (guanine nucleotide exchange protein)
Contig52405_RC	-0.470705		ESTs, Weakly similar to ALU8_HUMAN ALU SUBFAMILY SX SEQUENCE CONTAMINATION WARNING ENTRY [H.sapiens]
Contig30092_RC	-0.469977		Homo sapiens PR-domain zinc finger protein 6 isoform B (PRDM6) mRNA, partial cds; alternatively spliced
NM_003462	-0.468753	P28	dynein, axonemal, light intermediate polypeptide
Contig60393	0.468475		ESTs
Contig842_RC	0.468158		ESTs
NM_002982	0.466362	SCYA2	small inducible cytokine A2 (monocyte chemotactic protein 1, homologous to mouse Sig-je)
Contig14390_RC	0.464150		ESTs
NM_001770	0.463847	CD19	CD19 antigen
AK000617	-0.463158		Homo sapiens mRNA; cDNA DKFZp434L235 (from clone DKFZp434L235)
AF073299	-0.463007	SLC9A2	solute carrier family 9 (sodium/hydrogen exchanger), isoform 2
NM_019049	0.461990	FLJ20054	hypothetical protein
AL137347	-0.460778	DKFZP761M1511	hypothetical protein
NM_000396	-0.460263	CTSK	cathepsin K (pycnodysostosis)
NM_018373	-0.459268	FLJ11271	hypothetical protein FLJ11271
NM_002709	0.458500	PPP1CB	protein phosphatase 1, catalytic subunit, beta isoform
NM_016820	0.457516	OGG1	8-oxoguanine DNA glycosylase
Contig10268_RC	0.456933		Human DNA sequence from clone RP11-196N14 on chromosome 20 Contains ESTs, STSs, GSSs and CpG islands. Contains three novel genes, part of a gene for a novel protein similar to protein serine/threonine phosphatase 4 regulatory subunit 1 (PP4R1) and a gene for a novel protein with an ankyrin domain

Identifier	Correlation	Sequence Name	Description
NM_014521	-0.456733	SH3BP4	SH3-domain binding protein 4
AJ272057	-0.456548	STRAIT11499	hypothetical protein STRAIT11499
NM_015964	-0.456187	LOC51673	brain specific protein
Contig16759_RC	-0.456169		ESTs
NM_015937	-0.455954	LOC51604	CGI-06 protein
NM_007246	-0.455500	KLHL2	kelch (Drosophila)-like 2 (Mayven)
NM_001985	-0.453024	ETFB	electron-transfer-flavoprotein, beta polypeptide
NM_000984	-0.452935	RPL23A	ribosomal protein L23a
Contig51953_RC	-0.451695		ESTs
NM_015984	0.450491	UCH37	ubiquitin C-terminal hydrolase UCH37
NM_000903	-0.450371	DIA4	diaphorase (NADH/NADPH) (cytochrome b-5 reductase)
NM_001797	-0.449862	CDH11	cadherin 11, type 2, OB-cadherin (osteoblast)
NM_014878	0.449818	KIAA0020	KIAA0020 gene product
NM_002742	-0.449590	PRKCM	protein kinase C, mu

Table 5. 231 gene markers that distinguish patients with good prognosis from patients with poor prognosis.

GenBank Accession Number	SEQ ID NO	GenBank Accession Number	SEQ ID NO
AA555029_RC	SEQ ID NO 1	NM_013296	SEQ ID NO 1427
AB020689	SEQ ID NO 37	NM_013437	SEQ ID NO 1439
AB032973	SEQ ID NO 55	NM_014078	SEQ ID NO 1449
AB033007	SEQ ID NO 58	NM_014109	SEQ ID NO 1451
AB033043	SEQ ID NO 62	NM_014321	SEQ ID NO 1477
AB037745	SEQ ID NO 75	NM_014363	SEQ ID NO 1480
AB037863	SEQ ID NO 88	NM_014750	SEQ ID NO 1527
AF052159	SEQ ID NO 120	NM_014754	SEQ ID NO 1528
AF052162	SEQ ID NO 121	NM_014791	SEQ ID NO 1535
AF055033	SEQ ID NO 124	NM_014875	SEQ ID NO 1545
AF073519	SEQ ID NO 137	NM_014889	SEQ ID NO 1548
AF148505	SEQ ID NO 169	NM_014968	SEQ ID NO 1554
AF155117	SEQ ID NO 173	NM_015416	SEQ ID NO 1559
AF161553	SEQ ID NO 177	NM_015417	SEQ ID NO 1560
AF201951	SEQ ID NO 183	NM_015434	SEQ ID NO 1562
AF257175	SEQ ID NO 189	NM_015984	SEQ ID NO 1587
AJ224741	SEQ ID NO 196	NM_016337	SEQ ID NO 1636
AK000745	SEQ ID NO 219	NM_016359	SEQ ID NO 1638
AL050021	SEQ ID NO 257	NM_016448	SEQ ID NO 1645
AL050090	SEQ ID NO 259	NM_016569	SEQ ID NO 1655
AL080059	SEQ ID NO 270	NM_016577	SEQ ID NO 1656
AL080079	SEQ ID NO 271	NM_017779	SEQ ID NO 1708
AL080110	SEQ ID NO 272	NM_018004	SEQ ID NO 1725
AL133603	SEQ ID NO 306	NM_018098	SEQ ID NO 1739
AL133619	SEQ ID NO 307	NM_018104	SEQ ID NO 1743
AL137295	SEQ ID NO 315	NM_018120	SEQ ID NO 1745
AL137502	SEQ ID NO 326	NM_018136	SEQ ID NO 1748
AL137514	SEQ ID NO 327	NM_018265	SEQ ID NO 1766
AL137718	SEQ ID NO 336	NM_018354	SEQ ID NO 1774
AL355708	SEQ ID NO 353	NM_018401	SEQ ID NO 1782
D25328	SEQ ID NO 357	NM_018410	SEQ ID NO 1783
L27560	SEQ ID NO 390	NM_018454	SEQ ID NO 1786
M21551	SEQ ID NO 394	NM_018455	SEQ ID NO 1787
NM_000017	SEQ ID NO 416	NM_019013	SEQ ID NO 1809
NM_000096	SEQ ID NO 430	NM_020166	SEQ ID NO 1825

GenBank Accession Number	SEQ ID NO	GenBank Accession Number	SEQ ID NO
NM_000127	SEQ ID NO 436	NM_020188	SEQ ID NO 1830
NM_000158	SEQ ID NO 442	NM_020244	SEQ ID NO 1835
NM_000224	SEQ ID NO 453	NM_020386	SEQ ID NO 1838
NM_000286	SEQ ID NO 462	NM_020675	SEQ ID NO 1842
NM_000291	SEQ ID NO 463	NM_020974	SEQ ID NO 1844
NM_000320	SEQ ID NO 469	R70506_RC	SEQ ID NO 1868
NM_000436	SEQ ID NO 487	U45975	SEQ ID NO 1878
NM_000507	SEQ ID NO 491	U58033	SEQ ID NO 1881
NM_000599	SEQ ID NO 503	U82987	SEQ ID NO 1891
NM_000788	SEQ ID NO 527	U96131	SEQ ID NO 1896
NM_000849	SEQ ID NO 530	X05610	SEQ ID NO 1903
NM_001007	SEQ ID NO 550	X94232	SEQ ID NO 1927
NM_001124	SEQ ID NO 562	Contig753_RC	SEQ ID NO 1954
NM_001168	SEQ ID NO 566	Contig1778_RC	SEQ ID NO 1979
NM_001216	SEQ ID NO 574	Contig2399_RC	SEQ ID NO 1989
NM_001280	SEQ ID NO 588	Contig2504_RC	SEQ ID NO 1991
NM_001282	SEQ ID NO 589	Contig3902_RC	SEQ ID NO 2017
NM_001333	SEQ ID NO 597	Contig4595	SEQ ID NO 2022
NM_001673	SEQ ID NO 645	Contig8581_RC	SEQ ID NO 2037
NM_001809	SEQ ID NO 673	Contig13480_RC	SEQ ID NO 2052
NM_001827	SEQ ID NO 676	Contig17359_RC	SEQ ID NO 2068
NM_001905	SEQ ID NO 691	Contig20217_RC	SEQ ID NO 2072
NM_002019	SEQ ID NO 711	Contig21812_RC	SEQ ID NO 2082
NM_002073	SEQ ID NO 721	Contig24252_RC	SEQ ID NO 2087
NM_002358	SEQ ID NO 764	Contig25055_RC	SEQ ID NO 2090
NM_002570	SEQ ID NO 787	Contig25343_RC	SEQ ID NO 2092
NM_002808	SEQ ID NO 822	Contig25991	SEQ ID NO 2098
NM_002811	SEQ ID NO 823	Contig27312_RC	SEQ ID NO 2108
NM_002900	SEQ ID NO 835	Contig28552_RC	SEQ ID NO 2120
NM_002916	SEQ ID NO 838	Contig32125_RC	SEQ ID NO 2155
NM_003158	SEQ ID NO 881	Contig32185_RC	SEQ ID NO 2156
NM_003234	SEQ ID NO 891	Contig33814_RC	SEQ ID NO 2169
NM_003239	SEQ ID NO 893	Contig34634_RC	SEQ ID NO 2180
NM_003258	SEQ ID NO 896	Contig35251_RC	SEQ ID NO 2185
NM_003376	SEQ ID NO 906	Contig37063_RC	SEQ ID NO 2206
NM_003600	SEQ ID NO 929	Contig37598	SEQ ID NO 2216
NM_003607	SEQ ID NO 930	Contig38288_RC	SEQ ID NO 2223

GenBank Accession Number	SEQ ID NO	GenBank Accession Number	SEQ ID NO
NM_003662	SEQ ID NO 938	Contig40128_RC	SEQ ID NO 2248
NM_003676	SEQ ID NO 941	Contig40831_RC	SEQ ID NO 2260
NM_003748	SEQ ID NO 951	Contig41413_RC	SEQ ID NO 2266
NM_003862	SEQ ID NO 960	Contig41887_RC	SEQ ID NO 2276
NM_003875	SEQ ID NO 962	Contig42421_RC	SEQ ID NO 2291
NM_003878	SEQ ID NO 963	Contig43747_RC	SEQ ID NO 2311
NM_003882	SEQ ID NO 964	Contig44064_RC	SEQ ID NO 2315
NM_003981	SEQ ID NO 977	Contig44289_RC	SEQ ID NO 2320
NM_004052	SEQ ID NO 985	Contig44799_RC	SEQ ID NO 2330
NM_004163	SEQ ID NO 995	Contig45347_RC	SEQ ID NO 2344
NM_004336	SEQ ID NO 1022	Contig45816_RC	SEQ ID NO 2351
NM_004358	SEQ ID NO 1026	Contig46218_RC	SEQ ID NO 2358
NM_004456	SEQ ID NO 1043	Contig46223_RC	SEQ ID NO 2359
NM_004480	SEQ ID NO 1046	Contig46653_RC	SEQ ID NO 2369
NM_004504	SEQ ID NO 1051	Contig46802_RC	SEQ ID NO 2372
NM_004603	SEQ ID NO 1064	Contig47405_RC	SEQ ID NO 2384
NM_004701	SEQ ID NO 1075	Contig48328_RC	SEQ ID NO 2400
NM_004702	SEQ ID NO 1076	Contig49670_RC	SEQ ID NO 2434
NM_004798	SEQ ID NO 1087	Contig50106_RC	SEQ ID NO 2445
NM_004911	SEQ ID NO 1102	Contig50410	SEQ ID NO 2453
NM_004994	SEQ ID NO 1108	Contig50802_RC	SEQ ID NO 2463
NM_005196	SEQ ID NO 1127	Contig51464_RC	SEQ ID NO 2481
NM_005342	SEQ ID NO 1143	Contig51519_RC	SEQ ID NO 2482
NM_005496	SEQ ID NO 1157	Contig51749_RC	SEQ ID NO 2486
NM_005563	SEQ ID NO 1173	Contig51963	SEQ ID NO 2494
NM_005915	SEQ ID NO 1215	Contig53226_RC	SEQ ID NO 2525
NM_006096	SEQ ID NO 1240	Contig53268_RC	SEQ ID NO 2529
NM_006101	SEQ ID NO 1241	Contig53646_RC	SEQ ID NO 2538
NM_006115	SEQ ID NO 1245	Contig53742_RC	SEQ ID NO 2542
NM_006117	SEQ ID NO 1246	Contig55188_RC	SEQ ID NO 2586
NM_006201	SEQ ID NO 1254	Contig55313_RC	SEQ ID NO 2590
NM_006265	SEQ ID NO 1260	Contig55377_RC	SEQ ID NO 2591
NM_006281	SEQ ID NO 1263	Contig55725_RC	SEQ ID NO 2600
NM_006372	SEQ ID NO 1273	Contig55813_RC	SEQ ID NO 2607
NM_006681	SEQ ID NO 1306	Contig55829_RC	SEQ ID NO 2608
NM_006763	SEQ ID NO 1315	Contig56457_RC	SEQ ID NO 2630
NM_006931	SEQ ID NO 1341	Contig57595	SEQ ID NO 2655

GenBank Accession Number	SEQ ID NO	GenBank Accession Number	SEQ ID NO
NM_007036	SEQ ID NO 1349	Contig57864_RC	SEQ ID NO 2663
NM_007203	SEQ ID NO 1362	Contig58368_RC	SEQ ID NO 2668
NM_012177	SEQ ID NO 1390	Contig60864_RC	SEQ ID NO 2676
NM_012214	SEQ ID NO 1392	Contig63102_RC	SEQ ID NO 2684
NM_012261	SEQ ID NO 1397	Contig63649_RC	SEQ ID NO 2686
NM_012429	SEQ ID NO 1413	Contig64688	SEQ ID NO 2690
NM_013262	SEQ ID NO 1425		

Table 6. 70 Preferred prognosis markers drawn from Table 5.

Identifier	Correlation	Sequence Name	Description
AL080059	-0.527150		Homo sapiens mRNA for KIAA1750 protein, partial cds
Contig63649_RC	-0.468130		ESTs
Contig46218_RC	-0.432540		ESTs
NM_016359	-0.424930	LOC51203	clone HQ0310 PRO0310p1
AA555029_RC	-0.424120		ESTs
NM_003748	0.420671	ALDH4	aldehyde dehydrogenase 4 (glutamate gamma-semialdehyde dehydrogenase; pyrroline-5-carboxylate dehydrogenase)
Contig38288_RC	-0.414970		ESTs, Weakly similar to ISHUSS protein disulfide-isomerase [H.sapiens]
NM_003862	0.410964	FGF18	fibroblast growth factor 18
Contig28552_RC	-0.409260		Homo sapiens mRNA; cDNA DKFZp434C0931 (from clone DKFZp434C0931); partial cds
Contig32125_RC	0.409054		ESTs
U82987	0.407002	BBC3	Bcl-2 binding component 3
AL137718	-0.404980		Homo sapiens mRNA; cDNA DKFZp434C0931 (from clone DKFZp434C0931); partial cds
AB037863	0.402335	KIAA1442	KIAA1442 protein
NM_020188	-0.400070	DC13	DC13 protein
NM_020974	0.399987	CEGP1	CEGP1 protein
NM_000127	-0.399520	EXT1	exostoses (multiple) 1
NM_002019	-0.398070	FLT1	fms-related tyrosine kinase 1 (vascular endothelial growth factor/vascular permeability factor receptor)
NM_002073	-0.395460	GNAZ	guanine nucleotide binding protein (G protein), alpha z polypeptide
NM_000436	-0.392120	OXCT	3-oxoacid CoA transferase
NM_004994	-0.391690	MMP9	matrix metalloproteinase 9 (gelatinase B, 92kD gelatinase, 92kD type IV collagenase)
Contig55377_RC	0.390600		ESTs

Contig35251_RC	-0.390410		Homo sapiens cDNA: FLJ22719 fis, clone HSI14307
Contig25991	-0.390370	ECT2	epithelial cell transforming sequence 2 oncogene
NM_003875	-0.386520	GMPS	guanine monphosphate synthetase
NM_006101	-0.385890	HEC	highly expressed in cancer, rich in leucine heptad repeats
NM_003882	0.384479	WISP1	WNT1 inducible signaling pathway protein 1
NM_003607	-0.384390	PK428	Ser-Thr protein kinase related to the myotonic dystrophy protein kinase
AF073519	-0.383340	SERF1A	small EDRK-rich factor 1A (telomeric)
AF052162	-0.380830	FLJ12443	hypothetical protein FLJ12443
NM_000849	0.380831	GSTM3	glutathione S-transferase M3 (brain)
Contig32185_RC	-0.379170		Homo sapiens cDNA FLJ13997 fis, clone Y79AA1002220
NM_016577	-0.376230	RAB6B	RAB6B, member RAS oncogene family
Contig48328_RC	0.375252		ESTs, Weakly similar to T17248 hypothetical protein DKFZp586G1122.1 [H.sapiens]
Contig46223_RC	0.374289		ESTs
NM_015984	-0.373880	UCH37	ubiquitin C-terminal hydrolase UCH37
NM_006117	0.373290	PECI	peroxisomal D3,D2-enoyl-CoA isomerase
AK000745	-0.373060		Homo sapiens cDNA FLJ20738 fis, clone HEP08257
Contig40831_RC	-0.372930		ESTs
NM_003239	0.371524	TGFB3	transforming growth factor, beta 3
NM_014791	-0.370860	KIAA0175	KIAA0175 gene product
X05610	-0.370860	COL4A2	collagen, type IV, alpha 2
NM_016448	-0.369420	L2DTL	L2DTL protein
NM_018401	0.368349	HSA250839	gene for serine/threonine protein kinase
NM_000788	-0.367700	DCK	deoxycytidine kinase
Contig51464_RC	-0.367450	FLJ22477	hypothetical protein FLJ22477
AL080079	-0.367390	DKFZP564D0462	hypothetical protein DKFZp564D0462

NM_006931	-0.366490	SLC2A3	solute carrier family 2 (facilitated glucose transporter), member 3
AF257175	0.365900		Homo sapiens hepatocellular carcinoma-associated antigen 64 (HCA64) mRNA, complete cds
NM_014321	-0.365810	ORC6L	origin recognition complex, subunit 6 (yeast homolog)-like
NM_002916	-0.365590	RFC4	replication factor C (activator 1) 4 (37kD)
Contig55725_RC	-0.365350		ESTs, Moderately similar to T50635 hypothetical protein DKFZp762L0311.1 [H.sapiens]
Contig24252_RC	-0.364990		ESTs
AF201951	0.363953	CFFM4	high affinity immunoglobulin epsilon receptor beta subunit
NM_005915	-0.363850	MCM6	minichromosome maintenance deficient (mis5, S. pombe) 6
NM_001282	0.363326	AP2B1	adaptor-related protein complex 2, beta 1 subunit
Contig56457_RC	-0.361650	TMEFF1	transmembrane protein with EGF-like and two follistatin-like domains 1
NM_000599	-0.361290	IGFBP5	insulin-like growth factor binding protein 5
NM_020386	-0.360780	LOC57110	H-REV107 protein-related protein
NM_014889	-0.360040	MP1	metalloprotease 1 (pitrilysin family)
AF055033	-0.359940	IGFBP5	insulin-like growth factor binding protein 5
NM_006681	-0.359700	NMU	neuromedin U
NM_007203	-0.359570	AKAP2	A kinase (PRKA) anchor protein 2
Contig63102_RC	0.359255	FLJ11354	hypothetical protein FLJ11354
NM_003981	-0.358260	PRC1	protein regulator of cytokinesis 1
Contig20217_RC	-0.357880		ESTs
NM_001809	-0.357720	CENPA	centromere protein A (17kD)
Contig2399_RC	-0.356600	SM-20	similar to rat smooth muscle protein SM-20
NM_004702	-0.356600	CCNE2	cyclin E2
NM_007036	-0.356540	ESM1	endothelial cell-specific molecule 1
NM_018354	-0.356000	FLJ11190	hypothetical protein FLJ11190

The sets of markers listed in Tables 1-6 partially overlap; in other words, some markers are present in multiple sets, while other markers are unique to a set (FIG. 1). Thus, in one embodiment, the invention provides a set of 256 genetic markers that can distinguish between ER(+) and ER(-), and also between *BRCA1* tumors and sporadic tumors (*i.e.*, classify a tumor as ER(-) or ER(-) and *BRCA1*-related or sporadic). In a more specific embodiment, the invention provides subsets of at least 20, at least 50, at least 100, or at least 150 of the set of 256 markers, that can classify a tumor as ER(-) or ER(-) and *BRCA1*-related or sporadic. In another embodiment, the invention provides 165 markers that can distinguish between ER(+) and ER(-), and also between patients with good versus poor prognosis (*i.e.*, classify a tumor as either ER(-) or ER(+) and as having been removed from a patient with a good prognosis or a poor prognosis). In a more specific embodiment, the invention further provides subsets of at least 20, 50, 100 or 125 of the full set of 165 markers, which also classify a tumor as either ER(-) or ER(+) and as having been removed from a patient with a good prognosis or a poor prognosis The invention further provides a set of twelve markers that can distinguish between *BRCA1* tumors and sporadic tumors, and between patients with good versus poor prognosis. Finally, the invention provides eleven markers capable of differentiating all three statuses. Conversely, the invention provides 2,050 of the 2,460 ER-status markers that can determine only ER status, 173 of the 430 *BRCA1* v. sporadic markers that can determine only *BRCA1* v. sporadic status, and 65 of the 231 prognosis markers that can only determine prognosis. In more specific embodiments, the invention also provides for subsets of at least 20, 50, 100, 200, 500, 1,000, 1,500 or 2,000 of the 2,050 ER-status markers that also determine only ER status. The invention also provides subsets of at least 20, 50, 100 or 150 of the 173 markers that also determine only *BRCA1* v. sporadic status. The invention further provides subsets of at least 20, 30, 40, or 50 of the 65 prognostic markers that also determine only prognostic status.

Any of the sets of markers provided above may be used alone specifically or in combination with markers outside the set. For example, markers that distinguish ER-status may be used in combination with the *BRCA1* vs. sporadic markers, or with the prognostic markers, or both. Any of the marker sets provided above may also be used in combination with other markers for breast cancer, or for any other clinical or physiological condition.

The relationship between the marker sets is diagramed in FIG. 1.

5.3.2 IDENTIFICATION OF MARKERS

The present invention provides sets of markers for the identification of conditions or indications associated with breast cancer. Generally, the marker sets were identified by determining which of ~25,000 human markers had expression patters that correlated with the conditions or indications.

In one embodiment, the method for identifying marker sets is as follows. After extraction and labeling of target polynucleotides, the expression of all markers (genes) in a sample X is compared to the expression of all markers in a standard or control. In one embodiment, the standard or control comprises target polynucleotide molecules derived from a sample from a normal individual (*i.e.*, an individual not afflicted with breast cancer). In a preferred embodiment, the standard or control is a pool of target polynucleotide molecules. The pool may derived from collected samples from a number of normal individuals. In a preferred embodiment, the pool comprises samples taken from a number of individuals having sporadic-type tumors. In another preferred embodiment, the pool comprises an artificially-generated population of nucleic acids designed to approximate the level of nucleic acid derived from each marker found in a pool of marker-derived nucleic acids derived from tumor samples. In yet another embodiment, the pool is derived from normal or breast cancer cell lines or cell line samples.

The comparison may be accomplished by any means known in the art. For example, expression levels of various markers may be assessed by separation of target polynucleotide molecules (*e.g.*, RNA or cDNA) derived from the markers in agarose or polyacrylamide gels, followed by hybridization with marker-specific oligonucleotide probes. Alternatively, the comparison may be accomplished by the labeling of target polynucleotide molecules followed by separation on a sequencing gel. Polynucleotide samples are placed on the gel such that patient and control or standard polynucleotides are in adjacent lanes. Comparison of expression levels is accomplished visually or by means of densitometer. In a preferred embodiment, the expression of all markers is assessed simultaneously by hybridization to a microarray. In each approach, markers meeting certain criteria are identified as associated with breast cancer.

A marker is selected based upon significant difference of expression in a sample as compared to a standard or control condition. Selection may be made based upon either significant up- or down regulation of the marker in the patient sample. Selection may also be made by calculation of the statistical significance (*i.e.*, the p-value) of the correlation between the expression of the marker and the condition or indication. Preferably, both selection criteria are used. Thus, in one embodiment of the present invention, markers

associated with breast cancer are selected where the markers show both more than two-fold change (increase or decrease) in expression as compared to a standard, and the p-value for the correlation between the existence of breast cancer and the change in marker expression is no more than 0.01 (*i.e.*, is statistically significant).

The expression of the identified breast cancer-related markers is then used to identify markers that can differentiate tumors into clinical types. In a specific embodiment using a number of tumor samples, markers are identified by calculation of correlation coefficients between the clinical category or clinical parameter(s) and the linear, logarithmic or any transform of the expression ratio across all samples for each individual gene. Specifically, the correlation coefficient is calculated as

$$\rho = (\vec{c} \bullet \vec{r}) / (\|\vec{c}\| \cdot \|\vec{r}\|) \qquad \text{Equation (2)}$$

where $\vec{c}$ represents the clinical parameters or categories and $\vec{r}$ represents the linear, logarithmic or any transform of the ratio of expression between sample and control. Markers for which the coefficient of correlation exceeds a cutoff are identified as breast cancer-related markers specific for a particular clinical type. Such a cutoff or threshold corresponds to a certain significance of discriminating genes obtained by Monte Carlo simulations. The threshold depends upon the number of samples used; the threshold can be calculated as 3 X $1/\sqrt{n-3}$, where $1/\sqrt{n-3}$ is the distribution width and n = the number of samples. In a specific embodiment, markers are chosen if the correlation coefficient is greater than about 0.3 or less than about -0.3.

Next, the significance of the correlation is calculated. This significance may be calculated by any statistical means by which such significance is calculated. In a specific example, a set of correlation data is generated using a Monte-Carlo technique to randomize the association between the expression difference of a particular marker and the clinical category. The frequency distribution of markers satisfying the criteria through calculation of correlation coefficients is compared to the number of markers satisfying the criteria in the data generated through the Monte-Carlo technique. The frequency distribution of markers satisfying the criteria in the Monte-Carlo runs is used to determine whether the number of markers selected by correlation with clinical data is significant. See Example 4.

Once a marker set is identified, the markers may be rank-ordered in order of significance of discrimination. One means of rank ordering is by the amplitude of correlation between the change in gene expression of the marker and the specific condition being discriminated. Another, preferred means is to use a statistical metric. In a specific embodiment, the metric is a Fisher-like statistic:

$$t = \frac{\left(\langle x_1 \rangle - \langle x_2 \rangle\right)}{\sqrt{\left[\sigma_1^2 (n_1 - 1) + \sigma_2^2 (n_2 - 1)\right] / (n_1 + n_2 - 1) / (1/n_1 + 1/n_2)}} \qquad \text{Equation (3)}$$

In this equation, $\langle x_1 \rangle$ is the error-weighted average of the log ratio of transcript expression measurements within a first diagnostic group (*e.g.*, ER(-), $\langle x_2 \rangle$ is the error-weighted average of log ratio within a second, related diagnostic group (*e.g.*, ER(+)), σ_1 is the variance of the log ratio within the ER(-) group and n_1 is the number of samples for which valid measurements of log ratios are available. σ_2 is the variance of log ratio within the second diagnostic group (*e.g.*, ER(+)), and n_2 is the number of samples for which valid measurements of log ratios are available. The *t*-value represents the variance-compensated difference between two means.

The rank-ordered marker set may be used to optimize the number of markers in the set used for discrimination. This is accomplished generally in a "leave one out" method as follows. In a first run, a subset, for example 5, of the markers from the top of the ranked list is used to generate a template, where out of X samples, X-1 are used to generate the template, and the status of the remaining sample is predicted. This process is repeated for every sample until every one of the X samples is predicted once. In a second run, additional markers, for example 5, are added, so that a template is now generated from 10 markers, and the outcome of the remaining sample is predicted. This process is repeated until the entire set of markers is used to generate the template. For each of the runs, type 1 error (false negative) and type 2 errors (false positive) are counted; the optimal number of markers is that number where the type 1 error rate, or type 2 error rate, or preferably the total of type 1 and type 2 error rate is lowest.

For prognostic markers, validation of the marker set may be accomplished by an additional statistic, a survival model. This statistic generates the probability of tumor distant metastases as a function of time since initial diagnosis. A number of models may be used, including Weibull, normal, log-normal, log logistic, log-exponential, or log-Rayleigh (Chapter 12 "Life Testing", S-PLUS 2000 GUIDE TO STATISTICS, Vol. 2, p. 368 (2000)). For the "normal" model, the probability of distant metastases P at time *t* is calculated as

$$P = \alpha \times \exp\left(-t^2/\tau^2\right) \qquad \text{Equation (4)}$$

where α is fixed and equal to 1, and τ is a parameter to be fitted and measures the "expected lifetime".

It will be apparent to those skilled in the art that the above methods, in particular the statistical methods, described above, are not limited to the identification of markers associated with breast cancer, but may be used to identify set of marker genes associated with any phenotype. The phenotype can be the presence or absence of a disease such as cancer, or the presence or absence of any identifying clinical condition associated with that cancer. In the disease context, the phenotype may be a prognosis such as a survival time, probability of distant metastases of a disease condition, or likelihood of a particular response to a therapeutic or prophylactic regimen. The phenotype need not be cancer, or a disease; the phenotype may be a nominal characteristic associated with a healthy individual.

5.3.3 SAMPLE COLLECTION

In the present invention, target polynucleotide molecules are extracted from a sample taken from an individual afflicted with breast cancer. The sample may be collected in any clinically acceptable manner, but must be collected such that marker-derived polynucleotides (*i.e.*, RNA) are preserved. mRNA or nucleic acids derived therefrom (*i.e.*, cDNA or amplified DNA) are preferably labeled distinguishably from standard or control polynucleotide molecules, and both are simultaneously or independently hybridized to a microarray comprising some or all of the markers or marker sets or subsets described above. Alternatively, mRNA or nucleic acids derived therefrom may be labeled with the same label as the standard or control polynucleotide molecules, wherein the intensity of hybridization of each at a particular probe is compared. A sample may comprise any clinically relevant tissue sample, such as a tumor biopsy or fine needle aspirate, or a sample of bodily fluid, such as blood, plasma, serum, lymph, ascitic fluid, cystic fluid, urine or nipple exudate. The sample may be taken from a human, or, in a veterinary context, from non-human animals such as ruminants, horses, swine or sheep, or from domestic companion animals such as felines and canines.

Methods for preparing total and poly(A)+ RNA are well known and are described generally in Sambrook *et al.*, MOLECULAR CLONING - A LABORATORY MANUAL (2ND ED.), Vols. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (1989)) and Ausubel *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, vol. 2, Current Protocols Publishing, New York (1994)).

RNA may be isolated from eukaryotic cells by procedures that involve lysis of the cells and denaturation of the proteins contained therein. Cells of interest include wild-type cells (*i.e.*, non-cancerous), drug-exposed wild-type cells, tumor- or tumor-derived cells, modified cells, normal or tumor cell line cells, and drug-exposed modified cells.

Additional steps may be employed to remove DNA. Cell lysis may be accomplished with a nonionic detergent, followed by microcentrifugation to remove the nuclei and hence the bulk of the cellular DNA. In one embodiment, RNA is extracted from cells of the various types of interest using guanidinium thiocyanate lysis followed by CsCl centrifugation to separate the RNA from DNA (Chirgwin *et al.*, *Biochemistry* 18:5294-5299 (1979)). Poly(A)+ RNA is selected by selection with oligo-dT cellulose (*see* Sambrook *et al.*, , MOLECULAR CLONING - A LABORATORY MANUAL (2ND ED.), Vols. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (1989). Alternatively, separation of RNA from DNA can be accomplished by organic extraction, for example, with hot phenol or phenol/chloroform/isoamyl alcohol.

If desired, RNase inhibitors may be added to the lysis buffer. Likewise, for certain cell types, it may be desirable to add a protein denaturation/digestion step to the protocol.

For many applications, it is desirable to preferentially enrich mRNA with respect to other cellular RNAs, such as transfer RNA (tRNA) and ribosomal RNA (rRNA). Most mRNAs contain a poly(A) tail at their 3' end. This allows them to be enriched by affinity chromatography, for example, using oligo(dT) or poly(U) coupled to a solid support, such as cellulose or Sephadex™ (see Ausubel et al., CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, vol. 2, Current Protocols Publishing, New York (1994). Once bound, poly(A)+ mRNA is eluted from the affinity column using 2 mM EDTA/0.1% SDS.

The sample of RNA can comprise a plurality of different mRNA molecules, each different mRNA molecule having a different nucleotide sequence. In a specific embodiment, the mRNA molecules in the RNA sample comprise at least 100 different nucleotide sequences. More preferably, the mRNA molecules of the RNA sample comprise mRNA molecules corresponding to each of the marker genes. In another specific embodiment, the RNA sample is a mammalian RNA sample.

In a specific embodiment, total RNA or mRNA from cells are used in the methods of the invention. The source of the RNA can be cells of a plant or animal, human, mammal, primate, non-human animal, dog, cat, mouse, rat, bird, yeast, eukaryote, prokaryote, etc. In specific embodiments, the method of the invention is used with a sample containing total mRNA or total RNA from 1 x 10^6 cells or less. In another embodiment,

proteins can be isolated from the foregoing sources, by methods known in the art, for use in expression analysis at the protein level.

Probes to the homologs of the marker sequences disclosed herein can be employed preferably wherein non-human nucleic acid is being assayed.

5.4 METHODS OF USING BREAST CANCER MARKER SETS

5.4.1 DIAGNOSTIC METHODS

The present invention provides for methods of using the marker sets to analyze a sample from an individual so as to determine the individual's tumor type or subtype at a molecular level, whether a tumor is of the ER(+) or ER(-) type, and whether the tumor is *BRCA1*-associated or sporadic. The individual need not actually be afflicted with breast cancer. Essentially, the expression of specific marker genes in the individual, or a sample taken therefrom, is compared to a standard or control. For example, assume two breast cancer-related conditions, X and Y. One can compare the level of expression of breast cancer prognostic markers for condition X in an individual to the level of the marker-derived polynucleotides in a control, wherein the level represents the level of expression exhibited by samples having condition X. In this instance, if the expression of the markers in the individual's sample is substantially (*i.e.*, statistically) different from that of the control, then the individual does not have condition X. Where, as here, the choice is bimodal (*i.e.*, a sample is either X or Y), the individual can additionally be said to have condition Y. Of course, the comparison to a control representing condition Y can also be performed. Preferably both are performed simultaneously, such that each control acts as both a positive and a negative control. The distinguishing result may thus either be a demonstrable difference from the expression levels (*i.e.*, the amount of marker-derived RNA, or polynucleotides derived therefrom) represented by the control, or no significant difference.

Thus, in one embodiment, the method of determining a particular tumor-related status of an individual comprises the steps of (1) hybridizing labeled target polynucleotides from an individual to a microarray containing one of the above marker sets; (2) hybridizing standard or control polynucleotides molecules to the microarray, wherein the standard or control molecules are differentially labeled from the target molecules; and (3) determining the difference in transcript levels, or lack thereof, between the target and standard or control, wherein the difference, or lack thereof, determines the individual's tumor-related status. In a more specific embodiment, the standard or control molecules comprise marker-derived polynucleotides from a pool of samples from normal individuals,

or a pool of tumor samples from individuals having sporadic-type tumors. In a preferred embodiment, the standard or control is an artificially-generated pool of marker-derived polynucleotides, which pool is designed to mimic the level of marker expression exhibited by clinical samples of normal or breast cancer tumor tissue having a particular clinical indication (*i.e.*, cancerous or non-cancerous; ER(+) or ER(-) tumor; *BRCA1*- or sporadic type tumor). In another specific embodiment, the control molecules comprise a pool derived from normal or breast cancer cell lines.

The present invention provides sets of markers useful for distinguishing ER(+) from ER(-) tumor types. Thus, in one embodiment of the above method, the level of polynucleotides (*i.e.*, mRNA or polynucleotides derived therefrom) in a sample from an individual, expressed from the markers provided in Table 1 are compared to the level of expression of the same markers from a control, wherein the control comprises marker-related polynucleotides derived from ER(+) samples, ER(-) samples, or both. Preferably, the comparison is to both ER(+) and ER(-), and preferably the comparison is to polynucleotide pools from a number of ER(+) and ER(-) samples, respectively. Where the individual's marker expression most closely resembles or correlates with the ER(+) control, and does not resemble or correlate with the ER(-) control, the individual is classified as ER(+). Where the pool is not pure ER(+) or ER(-), for example, a sporadic pool is used. A set of experiments using individuals with known ER status should be hybridized against the pool, in order to define the expression templates for the ER(+) and ER(-) group. Each individual with unknown ER status is hybridized against the same pool and the expression profile is compared to the templates (s) to determine the individual's ER status.

The present invention provides sets of markers useful for distinguishing *BRCA1*-related tumors from sporadic tumors. Thus, the method can be performed substantially as for the ER(+/-) determination, with the exception that the markers are those listed in Tables 3 and 4, and the control markers are a pool of marker-derived polynucleotides *BRCA1* tumor samples, and a pool of marker-derived polynucleotides from sporadic tumors. A patient is determined to have a *BRCA1* germline mutation where the expression of the individual's marker-derived polynucleotides most closely resemble, or are most closely correlated with, that of the *BRCA1* control. Where the control is not pure *BRCA1* or sporadic, two templates can be defined in a manner similar to that for ER status, as described above.

For the above two embodiments of the method, the full set of markers may be used (*i.e.*, the complete set of markers for Tables 1 or 3). In other embodiments, subsets

of the markers may be used. In a preferred embodiment, the preferred markers listed in Tables 2 or 4 are used.

The similarity between the marker expression profile of an individual and that of a control can be assessed a number of ways. In the simplest case, the profiles can be compared visually in a printout of expression difference data. Alternatively, the similarity can be calculated mathematically.

In one embodiment, the similarity measure between two patients x and y, or patient x and a template y, can be calculated using the following equation:

$$S = 1 - \left[\sum_{i=1}^{N_V} \frac{(x_i - \bar{x})}{\sigma_{x_i}} \frac{(y_i - \bar{y})}{\sigma_{y_i}} \Bigg/ \sqrt{\sum_{i=1}^{N_V} \left(\frac{x_i - \bar{x}}{\sigma_{x_i}} \right)^2 \sum_{i=1}^{N_V} \left(\frac{y_i - \bar{y}}{\sigma_{y_i}} \right)^2} \right] \qquad \text{Equation (5)}$$

In this equation, x and y are two patients with components of log ratio x_i and y_i, $i = 1,...,N = 4{,}986$. Associated with every value x_i is error σ_{x_i}. The smaller the value σ_{x_i}, the more reliable the measurement x_i. $\bar{x} = \sum_{i=1}^{N_V} \frac{x_i}{\sigma_{x_i}^2} \Big/ \sum_{i=1}^{N_V} \frac{1}{\sigma_{x_i}^2}$ is the error-weighted arithmetic mean.

In a preferred embodiment, templates are developed for sample comparison. The template is defined as the error-weighted log ratio average of the expression difference for the group of marker genes able to differentiate the particular breast cancer-related condition. For example, templates are defined for ER(+) samples and for ER(-) samples. Next, a classifier parameter is calculated. This parameter may be calculated using either expression level differences between the sample and template, or by calculation of a correlation coefficient. Such a coefficient, P_i, can be calculated using the following equation:

$$P_i = (\vec{z}_i \bullet \vec{y}) / (\|\vec{z}_i\| \cdot \|\vec{y}\|) \qquad \text{Equation (1)}$$

where z_i is the expression template i, and y is the expression profile of a patient.

Thus, in a more specific embodiment, the above method of determining a particular tumor-related status of an individual comprises the steps of (1) hybridizing

labeled target polynucleotides from an individual to a microarray containing one of the above marker sets; (2) hybridizing standard or control polynucleotides molecules to the microarray, wherein the standard or control molecules are differentially labeled from the target molecules; and (3) determining the ratio (or difference) of transcript levels between two channels (individual and control), or simply the transcript levels of the individual; and (4) comparing the results from (3) to the predefined templates, wherein said determining is accomplished by means of the statistic of Equation 1 or Equation 5, and wherein the difference, or lack thereof, determines the individual's tumor-related status.

5.4.2 PROGNOSTIC METHODS

The present invention provides sets of markers useful for distinguishing samples from those patients with a good prognosis from samples from patients with a poor prognosis. Thus, the invention further provides a method for using these markers to determine whether an individual afflicted with breast cancer will have a good or poor clinical prognosis. In one embodiment, the invention provides for method of determining whether an individual afflicted with breast cancer will likely experience a relapse within five years of initial diagnosis (*i.e.*, whether an individual has a poor prognosis) comprising (1) comparing the level of expression of the markers listed in Table 5 in a sample taken from the individual to the level of the same markers in a standard or control, where the standard or control levels represent those found in an individual with a poor prognosis; and (2) determining whether the level of the marker-related polynucleotides in the sample from the individual is significantly different than that of the control, wherein if no substantial difference is found, the patient has a poor prognosis, and if a substantial difference is found, the patient has a good prognosis. Persons of skill in the art will readily see that the markers associated with good prognosis can also be used as controls. In a more specific embodiment, both controls are run. In case the pool is not pure 'good prognosis' or 'poor prognosis', a set of experiments of individuals with known outcome should be hybridized against the pool to define the expression templates for the good prognosis and poor prognosis group. Each individual with unknown outcome is hybridized against the same pool and the resulting expression profile is compared to the templates to predict its outcome.

Poor prognosis of breast cancer may indicate that a tumor is relatively aggressive, while good prognosis may indicate that a tumor is relatively nonaggressive. Therefore, the invention provides for a method of determining a course of treatment of a breast cancer patient, comprising determining whether the level of expression of the 231

markers of Table 5, or a subset thereof, correlates with the level of these markers in a sample representing a good prognosis expression pattern or a poor prognosis pattern; and determining a course of treatment, wherein if the expression correlates with the poor prognosis pattern, the tumor is treated as an aggressive tumor.

As with the diagnostic markers, the method can use the complete set of markers listed in Table 5. However, subsets of the markers may also be used. In a preferred embodiment, the subset listed in Table 6 is used.

Classification of a sample as "good prognosis" or "poor prognosis" is accomplished substantially as for the diagnostic markers described above, wherein a template is generated to which the marker expression levels in the sample are compared.

The use of marker sets is not restricted to the prognosis of breast cancer-related conditions, and may be applied in a variety of phenotypes or conditions, clinical or experimental, in which gene expression plays a role. Where a set of markers has been identified that corresponds to two or more phenotypes, the marker sets can be used to distinguish these phenotypes. For example, the phenotypes may be the diagnosis and/or prognosis of clinical states or phenotypes associated with other cancers, other disease conditions, or other physiological conditions, wherein the expression level data is derived from a set of genes correlated with the particular physiological or disease condition.

5.4.3 IMPROVING SENSITIVITY TO EXPRESSION LEVEL DIFFERENCES

In using the markers disclosed herein, and, indeed, using any sets of markers to differentiate an individual having one phenotype from another individual having a second phenotype, one can compare the absolute expression of each of the markers in a sample to a control; for example, the control can be the average level of expression of each of the markers, respectively, in a pool of individuals. To increase the sensitivity of the comparison, however, the expression level values are preferably transformed in a number of ways.

For example, the expression level of each of the markers can be normalized by the average expression level of all markers the expression level of which is determined, or by the average expression level of a set of control genes. Thus, in one embodiment, the markers are represented by probes on a microarray, and the expression level of each of the markers is normalized by the mean or median expression level across all of the genes represented on the microarray, including any non-marker genes. In a specific embodiment, the normalization is carried out by dividing the median or mean level of expression of all of the genes on the microarray. In another embodiment, the expression levels of the markers is

normalized by the mean or median level of expression of a set of control markers. In a specific embodiment, the control markers comprise a set of housekeeping genes. In another specific embodiment, the normalization is accomplished by dividing by the median or mean expression level of the control genes.

The sensitivity of a marker-based assay will also be increased if the expression levels of individual markers are compared to the expression of the same markers in a pool of samples. Preferably, the comparison is to the mean or median expression level of each the marker genes in the pool of samples. Such a comparison may be accomplished, for example, by dividing by the mean or median expression level of the pool for each of the markers from the expression level each of the markers in the sample. This has the effect of accentuating the relative differences in expression between markers in the sample and markers in the pool as a whole, making comparisons more sensitive and more likely to produce meaningful results that the use of absolute expression levels alone. The expression level data may be transformed in any convenient way; preferably, the expression level data for all is log transformed before means or medians are taken.

In performing comparisons to a pool, two approaches may be used. First, the expression levels of the markers in the sample may be compared to the expression level of those markers in the pool, where nucleic acid derived from the sample and nucleic acid derived from the pool are hybridized during the course of a single experiment. Such an approach requires that new pool nucleic acid be generated for each comparison or limited numbers of comparisons, and is therefore limited by the amount of nucleic acid available. Alternatively, and preferably, the expression levels in a pool, whether normalized and/or transformed or not, are stored on a computer, or on computer-readable media, to be used in comparisons to the individual expression level data from the sample (*i.e.*, single-channel data).

Thus, the current invention provides the following method of classifying a first cell or organism as having one of at least two different phenotypes, where the different phenotypes comprise a first phenotype and a second phenotype. The level of expression of each of a plurality of genes in a first sample from the first cell or organism is compared to the level of expression of each of said genes, respectively, in a pooled sample from a plurality of cells or organisms, the plurality of cells or organisms comprising different cells or organisms exhibiting said at least two different phenotypes, respectively, to produce a first compared value. The first compared value is then compared to a second compared value, wherein said second compared value is the product of a method comprising comparing the level of expression of each of said genes in a sample from a cell or organism

characterized as having said first phenotype to the level of expression of each of said genes, respectively, in the pooled sample. The first compared value is then compared to a third compared value, wherein said third compared value is the product of a method comprising comparing the level of expression of each of the genes in a sample from a cell or organism characterized as having the second phenotype to the level of expression of each of the genes, respectively, in the pooled sample. Optionally, the first compared value can be compared to additional compared values, respectively, where each additional compared value is the product of a method comprising comparing the level of expression of each of said genes in a sample from a cell or organism characterized as having a phenotype different from said first and second phenotypes but included among the at least two different phenotypes, to the level of expression of each of said genes, respectively, in said pooled sample. Finally, a determination is made as to which of said second, third, and, if present, one or more additional compared values, said first compared value is most similar, wherein the first cell or organism is determined to have the phenotype of the cell or organism used to produce said compared value most similar to said first compared value.

In a specific embodiment of this method, the compared values are each ratios of the levels of expression of each of said genes. In another specific embodiment, each of the levels of expression of each of the genes in the pooled sample are normalized prior to any of the comparing steps. In a more specific embodiment, the normalization of the levels of expression is carried out by dividing by the median or mean level of the expression of each of the genes or dividing by the mean or median level of expression of one or more housekeeping genes in the pooled sample from said cell or organism. In another specific embodiment, the normalized levels of expression are subjected to a log transform, and the comparing steps comprise subtracting the log transform from the log of the levels of expression of each of the genes in the sample. In another specific embodiment, the two or more different phenotypes are different stages of a disease or disorder. In still another specific embodiment, the two or more different phenotypes are different prognoses of a disease or disorder. In yet another specific embodiment, the levels of expression of each of the genes, respectively, in the pooled sample or said levels of expression of each of said genes in a sample from the cell or organism characterized as having the first phenotype, second phenotype, or said phenotype different from said first and second phenotypes, respectively, are stored on a computer or on a computer-readable medium.

In another specific embodiment, the two phenotypes are ER(+) or ER(-) status. In another specific embodiment, the two phenotypes are *BRCA1* or sporadic tumor-

type status. In yet another specific embodiment, the two phenotypes are good prognosis and poor prognosis.

Of course, single-channel data may also be used without specific comparison to a mathematical sample pool. For example, a sample may be classified as having a first or a second phenotype, wherein the first and second phenotypes are related, by calculating the similarity between the expression of at least 5 markers in the sample, where the markers are correlated with the first or second phenotype, to the expression of the same markers in a first phenotype template and a second phenotype template, by (a) labeling nucleic acids derived from a sample with a fluorophore to obtain a pool of fluorophore-labeled nucleic acids; (b) contacting said fluorophore-labeled nucleic acid with a microarray under conditions such that hybridization can occur, detecting at each of a plurality of discrete loci on the microarray a flourescent emission signal from said fluorophore-labeled nucleic acid that is bound to said microarray under said conditions; and (c) determining the similarity of marker gene expression in the individual sample to the first and second templates, wherein if said expression is more similar to the first template, the sample is classified as having the first phenotype, and if said expression is more similar to the second template, the sample is classified as having the second phenotype.

5.5 DETERMINATION OF MARKER GENE EXPRESSION LEVELS

5.5.1 METHODS

The expression levels of the marker genes in a sample may be determined by any means known in the art. The expression level may be determined by isolating and determining the level (*i.e.*, amount) of nucleic acid transcribed from each marker gene. Alternatively, or additionally, the level of specific proteins translated from mRNA transcribed from a marker gene may be determined.

The level of expression of specific marker genes can be accomplished by determining the amount of mRNA, or polynucleotides derived therefrom, present in a sample. Any method for determining RNA levels can be used. For example, RNA is isolated from a sample and separated on an agarose gel. The separated RNA is then transferred to a solid support, such as a filter. Nucleic acid probes representing one or more markers are then hybridized to the filter by northern hybridization, and the amount of marker-derived RNA is determined. Such determination can be visual, or machine-aided, for example, by use of a densitometer. Another method of determining RNA levels is by use of a dot-blot or a slot-blot. In this method, RNA, or nucleic acid derived therefrom, from a sample is labeled. The RNA or nucleic acid derived therefrom is then hybridized to

a filter containing oligonucleotides derived from one or more marker genes, wherein the oligonucleotides are placed upon the filter at discrete, easily-identifiable locations. Hybridization, or lack thereof, of the labeled RNA to the filter-bound oligonucleotides is determined visually or by densitometer. Polynucleotides can be labeled using a radiolabel or a fluorescent (*i.e.*, visible) label.

These examples are not intended to be limiting; other methods of determining RNA abundance are known in the art.

The level of expression of particular marker genes may also be assessed by determining the level of the specific protein expressed from the marker genes. This can be accomplished, for example, by separation of proteins from a sample on a polyacrylamide gel, followed by identification of specific marker-derived proteins using antibodies in a western blot. Alternatively, proteins can be separated by two-dimensional gel electrophoresis systems. Two-dimensional gel electrophoresis is well-known in the art and typically involves isoelectric focusing along a first dimension followed by SDS-PAGE electrophoresis along a second dimension. *See, e.g.*, Hames et al, 1990, GEL ELECTROPHORESIS OF PROTEINS: A PRACTICAL APPROACH, IRL Press, New York; Shevchenko *et al.*, *Proc. Nat'l Acad. Sci.* USA 93:1440-1445 (1996); Sagliocco *et al.*, *Yeast* 12:1519-1533 (1996); Lander, *Science* 274:536-539 (1996). The resulting electropherograms can be analyzed by numerous techniques, including mass spectrometric techniques, western blotting and immunoblot analysis using polyclonal and monoclonal antibodies.

Alternatively, marker-derived protein levels can be determined by constructing an antibody microarray in which binding sites comprise immobilized, preferably monoclonal, antibodies specific to a plurality of protein species encoded by the cell genome. Preferably, antibodies are present for a substantial fraction of the marker-derived proteins of interest. Methods for making monoclonal antibodies are well known (*see, e.g.*, Harlow and Lane, 1988, ANTIBODIES: A LABORATORY MANUAL, Cold Spring Harbor, New York, which is incorporated in its entirety for all purposes). In one embodiment, monoclonal antibodies are raised against synthetic peptide fragments designed based on genomic sequence of the cell. With such an antibody array, proteins from the cell are contacted to the array. and their binding is assayed with assays known in the art. Generally, the expression, and the level of expression, of proteins of diagnostic or prognostic interest can be detected through immunohistochemical staining of tissue slices or sections.

Finally, expression of marker genes in a number of tissue specimens may be characterized using a "tissue array" (Kononen *et al.*, *Nat. Med* 4(7):844-7 (1998)). In a tissue array, multiple tissue samples are assessed on the same microarray. The arrays allow *in situ* detection of RNA and protein levels; consecutive sections allow the analysis of multiple samples simultaneously.

5.5.2 MICROARRAYS

In preferred embodiments, polynucleotide microarrays are used to measure expression so that the expression status of each of the markers above is assessed simultaneously. In a specific embodiment, the invention provides for oligonucleotide or cDNA arrays comprising probes hybridizable to the genes corresponding to each of the marker sets described above (*i.e.*, markers to determine the molecular type or subtype of a tumor; markers to distinguish ER status; markers to distinguish *BRCA1* from sporadic tumors; markers to distinguish patients with good versus patients with poor prognosis; markers to distinguish both ER(+) from ER(-), and *BRCA1* tumors from sporadic tumors; markers to distinguish ER(+) from ER(-), and patients with good prognosis from patients with poor prognosis; markers to distinguish *BRCA1* tumors from sporadic tumors, and patients with good prognosis from patients with poor prognosis; and markers able to distinguish ER(+) from ER(-), *BRCA1* tumors from sporadic tumors, and patients with good prognosis from patients with poor prognosis; and markers unique to each status).

The microarrays provided by the present invention may comprise probes hybridizable to the genes corresponding to markers able to distinguish the status of one, two, or all three of the clinical conditions noted above. In particular, the invention provides polynucleotide arrays comprising probes to a subset or subsets of at least 50, 100, 200, 300, 400, 500, 750, 1,000, 1,250, 1,500, 1,750, 2,000 or 2,250 genetic markers, up to the full set of 2,460 markers, which distinguish ER(+) and ER(-) patients or tumors. The invention also provides probes to subsets of at least 20, 30, 40, 50, 75, 100, 150, 200, 250, 300, 350 or 400 markers, up to the full set of 430 markers, which distinguish between tumors containing a *BRCA1* mutation and sporadic tumors within an ER(-) group of tumors. The invention also provides probes to subsets of at least 20, 30, 40, 50, 75, 100, 150 or 200 markers, up to the full set of 231 markers, which distinguish between patients with good and poor prognosis within sporadic tumors. In a specific embodiment, the array comprises probes to marker sets or subsets directed to any two of the clinical conditions. In a more specific embodiment, the array comprises probes to marker sets or subsets directed to all three clinical conditions.

In yet another specific embodiment, microarrays that are used in the methods disclosed herein optionally comprise markers additional to at least some of the markers listed in Tables 1-6. For example, in a specific embodiment, the microarray is a screening or scanning array as described in Altschuler *et al.*, International Publication WO 02/18646, published March 7, 2002 and Scherer *et al.*, International Publication WO 02/16650, published February 28, 2002. The scanning and screening arrays comprise regularly-spaced, positionally-addressable probes derived from genomic nucleic acid sequence, both expressed and unexpressed. Such arrays may comprise probes corresponding to a subset of, or all of, the markers listed in Tables 1-6, or a subset thereof as described above, and can be used to monitor marker expression in the same way as a microarray containing only markers listed in Tables 1-6.

In yet another specific embodiment, the microarray is a commercially-available cDNA microarray that comprises at least five of the markers listed in Tables 1-6. Preferably, a commercially-available cDNA microarray comprises all of the markers listed in Tables 1-6. However, such a microarray may comprise 5, 10, 15, 25, 50, 100, 150, 250, 500, 1000 or more of the markers in any of Tables 1-6, up to the maximum number of markers in a Table, and may comprise all of the markers in any one of Tables 1-6 and a subset of another of Tables 1-6, or subsets of each as described above. In a specific embodiment of the microarrays used in the methods disclosed herein, the markers that are all or a portion of Tables 1-6 make up at least 50%, 60%, 70%, 80%, 90%, 95% or 98% of the probes on the microarray.

General methods pertaining to the construction of microarrays comprising the marker sets and/or subsets above are described in the following sections.

5.5.2.1 CONSTRUCTION OF MICROARRAYS

Microarrays are prepared by selecting probes which comprise a polynucleotide sequence, and then immobilizing such probes to a solid support or surface. For example, the probes may comprise DNA sequences, RNA sequences, or copolymer sequences of DNA and RNA. The polynucleotide sequences of the probes may also comprise DNA and/or RNA analogues, or combinations thereof. For example, the polynucleotide sequences of the probes may be full or partial fragments of genomic DNA. The polynucleotide sequences of the probes may also be synthesized nucleotide sequences, such as synthetic oligonucleotide sequences. The probe sequences can be synthesized either enzymatically *in vivo*, enzymatically *in vitro* (*e.g.*, by PCR), or non-enzymatically *in vitro*.

The probe or probes used in the methods of the invention are preferably immobilized to a solid support which may be either porous or non-porous. For example, the probes of the invention may be polynucleotide sequences which are attached to a nitrocellulose or nylon membrane or filter covalently at either the 3' or the 5' end of the polynucleotide. Such hybridization probes are well known in the art (see, *e.g.*, Sambrook et al., MOLECULAR CLONING - A LABORATORY MANUAL (2ND ED.), Vols. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (1989). Alternatively, the solid support or surface may be a glass or plastic surface. In a particularly preferred embodiment, hybridization levels are measured to microarrays of probes consisting of a solid phase on the surface of which are immobilized a population of polynucleotides, such as a population of DNA or DNA mimics, or, alternatively, a population of RNA or RNA mimics. The solid phase may be a nonporous or, optionally, a porous material such as a gel.

In preferred embodiments, a microarray comprises a support or surface with an ordered array of binding (*e.g.*, hybridization) sites or "probes" each representing one of the markers described herein. Preferably the microarrays are addressable arrays, and more preferably positionally addressable arrays. More specifically, each probe of the array is preferably located at a known, predetermined position on the solid support such that the identity (*i.e.*, the sequence) of each probe can be determined from its position in the array (*i.e.*, on the support or surface). In preferred embodiments, each probe is covalently attached to the solid support at a single site.

Microarrays can be made in a number of ways, of which several are described below. However produced, microarrays share certain characteristics. The arrays are reproducible, allowing multiple copies of a given array to be produced and easily compared with each other. Preferably, microarrays are made from materials that are stable under binding (*e.g.*, nucleic acid hybridization) conditions. The microarrays are preferably small, *e.g.*, between 1 cm^2 and 25 cm^2, between 12 cm^2 and 13 cm^2, or 3 cm^2. However, larger arrays are also contemplated and may be preferable, *e.g.*, for use in screening arrays. Preferably, a given binding site or unique set of binding sites in the microarray will specifically bind (*e.g.*, hybridize) to the product of a single gene in a cell (*e.g.*, to a specific mRNA, or to a specific cDNA derived therefrom). However, in general, other related or similar sequences will cross hybridize to a given binding site.

The microarrays of the present invention include one or more test probes, each of which has a polynucleotide sequence that is complementary to a subsequence of RNA or DNA to be detected. Preferably, the position of each probe on the solid surface is known. Indeed, the microarrays are preferably positionally addressable arrays. Specifically, each

probe of the array is preferably located at a known, predetermined position on the solid support such that the identity (*i.e.*, the sequence) of each probe can be determined from its position on the array (*i.e.*, on the support or surface).

According to the invention, the microarray is an array (*i.e.*, a matrix) in which each position represents one of the markers described herein. For example, each position can contain a DNA or DNA analogue based on genomic DNA to which a particular RNA or cDNA transcribed from that genetic marker can specifically hybridize. The DNA or DNA analogue can be, *e.g.*, a synthetic oligomer or a gene fragment. In one embodiment, probes representing each of the markers is present on the array. In a preferred embodiment, the array comprises the 550 of the 2,460 RE-status markers, 70 of the *BRCA1*/sporadic markers, and all 231 of the prognosis markers.

5.5.2.2 PREPARING PROBES FOR MICROARRAYS

As noted above, the "probe" to which a particular polynucleotide molecule specifically hybridizes according to the invention contains a complementary genomic polynucleotide sequence. The probes of the microarray preferably consist of nucleotide sequences of no more than 1,000 nucleotides. In some embodiments, the probes of the array consist of nucleotide sequences of 10 to 1,000 nucleotides. In a preferred embodiment, the nucleotide sequences of the probes are in the range of 10-200 nucleotides in length and are genomic sequences of a species of organism, such that a plurality of different probes is present, with sequences complementary and thus capable of hybridizing to the genome of such a species of organism, sequentially tiled across all or a portion of such genome. In other specific embodiments, the probes are in the range of 10-30 nucleotides in length, in the range of 10-40 nucleotides in length, in the range of 20-50 nucleotides in length, in the range of 40-80 nucleotides in length, in the range of 50-150 nucleotides in length, in the range of 80-120 nucleotides in length, and most preferably are 60 nucleotides in length.

The probes may comprise DNA or DNA "mimics" (*e.g.*, derivatives and analogues) corresponding to a portion of an organism's genome. In another embodiment, the probes of the microarray are complementary RNA or RNA mimics. DNA mimics are polymers composed of subunits capable of specific, Watson-Crick-like hybridization with DNA, or of specific hybridization with RNA. The nucleic acids can be modified at the base moiety, at the sugar moiety, or at the phosphate backbone. Exemplary DNA mimics include, *e.g.*, phosphorothioates.

DNA can be obtained, *e.g.*, by polymerase chain reaction (PCR) amplification of genomic DNA or cloned sequences. PCR primers are preferably chosen based on a known

sequence of the genome that will result in amplification of specific fragments of genomic DNA. Computer programs that are well known in the art are useful in the design of primers with the required specificity and optimal amplification properties, such as *Oligo* version 5.0 (National Biosciences). Typically each probe on the microarray will be between 10 bases and 50,000 bases, usually between 300 bases and 1,000 bases in length. PCR methods are well known in the art, and are described, for example, in Innis *et al.*, eds., PCR PROTOCOLS: A GUIDE TO METHODS AND APPLICATIONS, Academic Press Inc., San Diego, CA (1990). It will be apparent to one skilled in the art that controlled robotic systems are useful for isolating and amplifying nucleic acids.

An alternative, preferred means for generating the polynucleotide probes of the microarray is by synthesis of synthetic polynucleotides or oligonucleotides, *e.g.*, using N-phosphonate or phosphoramidite chemistries (Froehler *et al.*, *Nucleic Acid Res.* 14:5399-5407 (1986); McBride *et al.*, *Tetrahedron Lett.* 24:246-248 (1983)). Synthetic sequences are typically between about 10 and about 500 bases in length, more typically between about 20 and about 100 bases, and most preferably between about 40 and about 70 bases in length. In some embodiments, synthetic nucleic acids include non-natural bases, such as, but by no means limited to, inosine. As noted above, nucleic acid analogues may be used as binding sites for hybridization. An example of a suitable nucleic acid analogue is peptide nucleic acid (*see, e.g.*, Egholm *et al.*, *Nature* 363:566-568 (1993); U.S. Patent No. 5,539,083). Probes are preferably selected using an algorithm that takes into account binding energies, base composition, sequence complexity, cross-hybridization binding energies, and secondary structure (*see* Friend *et al.*, International Patent Publication WO 01/05935, published January 25, 2001; Hughes *et al.*, *Nat. Biotech.* 19:342-7 (2001)).

A skilled artisan will also appreciate that positive control probes, *e.g.*, probes known to be complementary and hybridizable to sequences in the target polynucleotide molecules, and negative control probes, *e.g.*, probes known to not be complementary and hybridizable to sequences in the target polynucleotide molecules, should be included on the array. In one embodiment, positive controls are synthesized along the perimeter of the array. In another embodiment, positive controls are synthesized in diagonal stripes across the array. In still another embodiment, the reverse complement for each probe is synthesized next to the position of the probe to serve as a negative control. In yet another embodiment, sequences from other species of organism are used as negative controls or as "spike-in" controls.

5.5.2.3 ATTACHING PROBES TO THE SOLID SURFACE

The probes are attached to a solid support or surface, which may be made, *e.g.*, from glass, plastic (*e.g.*, polypropylene, nylon), polyacrylamide, nitrocellulose, gel, or other porous or nonporous material. A preferred method for attaching the nucleic acids to a surface is by printing on glass plates, as is described generally by Schena *et al*, *Science* 270:467-470 (1995). This method is especially useful for preparing microarrays of cDNA (See also, DeRisi *et al*, *Nature Genetics* 14:457-460 (1996); Shalon *et al.*, *Genome Res.* 6:639-645 (1996); and Schena *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 93:10539-11286 (1995)).

A second preferred method for making microarrays is by making high-density oligonucleotide arrays. Techniques are known for producing arrays containing thousands of oligonucleotides complementary to defined sequences, at defined locations on a surface using photolithographic techniques for synthesis *in situ* (see, Fodor *et al.*, 1991, *Science* 251:767-773; Pease *et al.*, 1994, *Proc. Natl. Acad. Sci. U.S.A.* 91:5022-5026; Lockhart *et al.*, 1996, *Nature Biotechnology* 14:1675; U.S. Patent Nos. 5,578,832; 5,556,752; and 5,510,270) or other methods for rapid synthesis and deposition of defined oligonucleotides (Blanchard *et al.*, *Biosensors & Bioelectronics* 11:687-690). When these methods are used, oligonucleotides (*e.g.*, 60-mers) of known sequence are synthesized directly on a surface such as a derivatized glass slide. Usually, the array produced is redundant, with several oligonucleotide molecules per RNA.

Other methods for making microarrays, *e.g.*, by masking (Maskos and Southern, 1992, *Nuc. Acids. Res.* 20:1679-1684), may also be used. In principle, and as noted *supra*, any type of array, for example, dot blots on a nylon hybridization membrane (see Sambrook *et al.*, MOLECULAR CLONING - A LABORATORY MANUAL (2ND ED.), Vols. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (1989)) could be used. However, as will be recognized by those skilled in the art, very small arrays will frequently be preferred because hybridization volumes will be smaller.

In one embodiment, the arrays of the present invention are prepared by synthesizing polynucleotide probes on a support. In such an embodiment, polynucleotide probes are attached to the support covalently at either the 3' or the 5' end of the polynucleotide.

In a particularly preferred embodiment, microarrays of the invention are manufactured by means of an ink jet printing device for oligonucleotide synthesis, *e.g.*, using the methods and systems described by Blanchard in U.S. Pat. No. 6,028,189; Blanchard *et al.*, 1996, *Biosensors and Bioelectronics* 11:687-690; Blanchard, 1998, in SYNTHETIC DNA ARRAYS IN GENETIC ENGINEERING, Vol. 20, J.K. Setlow, Ed., Plenum Press, New York at pages 111-123. Specifically, the oligonucleotide probes in such

microarrays are preferably synthesized in arrays, *e.g.*, on a glass slide, by serially depositing individual nucleotide bases in "microdroplets" of a high surface tension solvent such as propylene carbonate. The microdroplets have small volumes (*e.g.*, 100 pL or less, more preferably 50 pL or less) and are separated from each other on the microarray (*e.g.*, by hydrophobic domains) to form circular surface tension wells which define the locations of the array elements (*i.e.*, the different probes). Microarrays manufactured by this ink-jet method are typically of high density, preferably having a density of at least about 2,500 different probes per 1 cm^2. The polynucleotide probes are attached to the support covalently at either the 3' or the 5' end of the polynucleotide.

5.5.2.4 TARGET POLYNUCLEOTIDE MOLECULES

The polynucleotide molecules which may be analyzed by the present invention (the "target polynucleotide molecules") may be from any clinically relevant source, but are expressed RNA or a nucleic acid derived therefrom (*e.g.*, cDNA or amplified RNA derived from cDNA that incorporates an RNA polymerase promoter), including naturally occurring nucleic acid molecules, as well as synthetic nucleic acid molecules. In one embodiment, the target polynucleotide molecules comprise RNA, including, but by no means limited to, total cellular RNA, poly(A)$^+$ messenger RNA (mRNA) or fraction thereof, cytoplasmic mRNA, or RNA transcribed from cDNA (*i.e.*, cRNA; see, *e.g.*, Linsley & Schelter, U.S. Patent Application No. 09/411,074, filed October 4, 1999, or U.S. Patent Nos. 5,545,522, 5,891,636, or 5,716,785). Methods for preparing total and poly(A)$^+$ RNA are well known in the art, and are described generally, *e.g.*, in Sambrook *et al.*, MOLECULAR CLONING - A LABORATORY MANUAL (2ND ED.), Vols. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (1989). In one embodiment, RNA is extracted from cells of the various types of interest in this invention using guanidinium thiocyanate lysis followed by CsCl centrifugation (Chirgwin *et al.*, 1979, *Biochemistry* 18:5294-5299). In another embodiment, total RNA is extracted using a silica gel-based column, commercially available examples of which include RNeasy (Qiagen, Valencia, California) and StrataPrep (Stratagene, La Jolla, California). In an alternative embodiment, which is preferred for *S. cerevisiae*, RNA is extracted from cells using phenol and chloroform, as described in Ausubel *et al.*, eds., 1989, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, Vol III, Green Publishing Associates, Inc., John Wiley & Sons, Inc., New York, at pp. 13.12.1-13.12.5). Poly(A)$^+$ RNA can be selected, *e.g.*, by selection with oligo-dT cellulose or, alternatively, by oligo-dT primed reverse transcription of total cellular RNA. In one embodiment, RNA can be fragmented by methods known in the art, *e.g.*, by incubation with $ZnCl_2$, to generate

fragments of RNA. In another embodiment, the polynucleotide molecules analyzed by the invention comprise cDNA, or PCR products of amplified RNA or cDNA.

In one embodiment, total RNA, mRNA, or nucleic acids derived therefrom, is isolated from a sample taken from a person afflicted with breast cancer. Target polynucleotide molecules that are poorly expressed in particular cells may be enriched using normalization techniques (Bonaldo *et al.*, 1996, *Genome Res.* 6:791-806).

As described above, the target polynucleotides are detectably labeled at one or more nucleotides. Any method known in the art may be used to detectably label the target polynucleotides. Preferably, this labeling incorporates the label uniformly along the length of the RNA, and more preferably, the labeling is carried out at a high degree of efficiency. One embodiment for this labeling uses oligo-dT primed reverse transcription to incorporate the label; however, conventional methods of this method are biased toward generating 3' end fragments. Thus, in a preferred embodiment, random primers (*e.g.*, 9-mers) are used in reverse transcription to uniformly incorporate labeled nucleotides over the full length of the target polynucleotides. Alternatively, random primers may be used in conjunction with PCR methods or T7 promoter-based *in vitro* transcription methods in order to amplify the target polynucleotides.

In a preferred embodiment, the detectable label is a luminescent label. For example, fluorescent labels, bio-luminescent labels, chemi-luminescent labels, and colorimetric labels may be used in the present invention. In a highly preferred embodiment, the label is a fluorescent label, such as a fluorescein, a phosphor, a rhodamine, or a polymethine dye derivative. Examples of commercially available fluorescent labels include, for example, fluorescent phosphoramidites such as FluorePrime (Amersham Pharmacia, Piscataway, N.J.), Fluoredite (Millipore, Bedford, Mass.), FAM (ABI, Foster City, Calif.), and Cy3 or Cy5 (Amersham Pharmacia, Piscataway, N.J.). In another embodiment, the detectable label is a radiolabeled nucleotide.

In a further preferred embodiment, target polynucleotide molecules from a patient sample are labeled differentially from target polynucleotide molecules of a standard. The standard can comprise target polynucleotide molecules from normal individuals (*i.e.*, those not afflicted with breast cancer). In a highly preferred embodiment, the standard comprises target polynucleotide molecules pooled from samples from normal individuals or tumor samples from individuals having sporadic-type breast tumors. In another embodiment, the target polynucleotide molecules are derived from the same individual, but are taken at different time points, and thus indicate the efficacy of a treatment by a change in expression of the markers, or lack thereof, during and after the course of treatment (*i.e.*, chemotherapy,

radiation therapy or cryotherapy), wherein a change in the expression of the markers from a poor prognosis pattern to a good prognosis pattern indicates that the treatment is efficacious. In this embodiment, different timepoints are differentially labeled.

5.5.2.5 HYBRIDIZATION TO MICROARRAYS

Nucleic acid hybridization and wash conditions are chosen so that the target polynucleotide molecules specifically bind or specifically hybridize to the complementary polynucleotide sequences of the array, preferably to a specific array site, wherein its complementary DNA is located.

Arrays containing double-stranded probe DNA situated thereon are preferably subjected to denaturing conditions to render the DNA single-stranded prior to contacting with the target polynucleotide molecules. Arrays containing single-stranded probe DNA (*e.g.*, synthetic oligodeoxyribonucleic acids) may need to be denatured prior to contacting with the target polynucleotide molecules, *e.g.*, to remove hairpins or dimers which form due to self complementary sequences.

Optimal hybridization conditions will depend on the length (*e.g.*, oligomer versus polynucleotide greater than 200 bases) and type (*e.g.*, RNA, or DNA) of probe and target nucleic acids. One of skill in the art will appreciate that as the oligonucleotides become shorter, it may become necessary to adjust their length to achieve a relatively uniform melting temperature for satisfactory hybridization results. General parameters for specific (*i.e.*, stringent) hybridization conditions for nucleic acids are described in Sambrook *et al.*, MOLECULAR CLONING - A LABORATORY MANUAL (2ND ED.), Vols. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (1989), and in Ausubel *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, vol. 2, Current Protocols Publishing, New York (1994). Typical hybridization conditions for the cDNA microarrays of Schena *et al.* are hybridization in 5 X SSC plus 0.2% SDS at 65 °C for four hours, followed by washes at 25 °C in low stringency wash buffer (1 X SSC plus 0.2% SDS), followed by 10 minutes at 25 °C in higher stringency wash buffer (0.1 X SSC plus 0.2% SDS) (Schena *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 93:10614 (1993)). Useful hybridization conditions are also provided in, *e.g.*, Tijessen, 1993, HYBRIDIZATION WITH NUCLEIC ACID PROBES, Elsevier Science Publishers B.V.; and Kricka, 1992, NONISOTOPIC DNA PROBE TECHNIQUES, Academic Press, San Diego, CA.

Particularly preferred hybridization conditions include hybridization at a temperature at or near the mean melting temperature of the probes (*e.g.*, within 5 °C, more preferably

within 2 °C) in 1 M NaCl, 50 mM MES buffer (pH 6.5), 0.5% sodium sarcosine and 30% formamide.

5.5.2.6 SIGNAL DETECTION AND DATA ANALYSIS

When fluorescently labeled probes are used, the fluorescence emissions at each site of a microarray may be, preferably, detected by scanning confocal laser microscopy. In one embodiment, a separate scan, using the appropriate excitation line, is carried out for each of the two fluorophores used. Alternatively, a laser may be used that allows simultaneous specimen illumination at wavelengths specific to the two fluorophores and emissions from the two fluorophores can be analyzed simultaneously (*see* Shalon *et al.*, 1996, "A DNA microarray system for analyzing complex DNA samples using two-color fluorescent probe hybridization," *Genome Research* 6:639-645, which is incorporated by reference in its entirety for all purposes). In a preferred embodiment, the arrays are scanned with a laser fluorescent scanner with a computer controlled X-Y stage and a microscope objective. Sequential excitation of the two fluorophores is achieved with a multi-line, mixed gas laser and the emitted light is split by wavelength and detected with two photomultiplier tubes. Fluorescence laser scanning devices are described in Schena *et al.*, *Genome Res.* 6:639-645 (1996), and in other references cited herein. Alternatively, the fiber-optic bundle described by Ferguson *et al.*, *Nature Biotech.* 14:1681-1684 (1996), may be used to monitor mRNA abundance levels at a large number of sites simultaneously.

Signals are recorded and, in a preferred embodiment, analyzed by computer, *e.g.*, using a 12 or 16 bit analog to digital board. In one embodiment the scanned image is despeckled using a graphics program (*e.g.*, Hijaak Graphics Suite) and then analyzed using an image gridding program that creates a spreadsheet of the average hybridization at each wavelength at each site. If necessary, an experimentally determined correction for "cross talk" (or overlap) between the channels for the two fluors may be made. For any particular hybridization site on the transcript array, a ratio of the emission of the two fluorophores can be calculated. The ratio is independent of the absolute expression level of the cognate gene, but is useful for genes whose expression is significantly modulated in association with the different breast cancer-related condition.

5.6 COMPUTER-FACILITATED ANALYSIS

The present invention further provides for kits comprising the marker sets above. In a preferred embodiment, the kit contains a microarray ready for hybridization to target polynucleotide molecules, plus software for the data analyses described above..

The analytic methods described in the previous sections can be implemented by use of the following computer systems and according to the following programs and methods. A Computer system comprises internal components linked to external components. The internal components of a typical computer system include a processor element interconnected with a main memory. For example, the computer system can be an Intel 8086-, 80386-, 80486-, Pentium™, or Pentium™-based processor with preferably 32 MB or more of main memory.

The external components may include mass storage. This mass storage can be one or more hard disks (which are typically packaged together with the processor and memory). Such hard disks are preferably of 1 GB or greater storage capacity. Other external components include a user interface device, which can be a monitor, together with an inputting device, which can be a "mouse", or other graphic input devices, and/or a keyboard. A printing device can also be attached to the computer.

Typically, a computer system is also linked to network link, which can be part of an Ethernet link to other local computer systems, remote computer systems, or wide area communication networks, such as the Internet. This network link allows the computer system to share data and processing tasks with other computer systems.

Loaded into memory during operation of this system are several software components, which are both standard in the art and special to the instant invention. These software components collectively cause the computer system to function according to the methods of this invention. These software components are typically stored on the mass storage device. A software component comprises the operating system, which is responsible for managing computer system and its network interconnections. This operating system can be, for example, of the Microsoft Windows® family, such as Windows 3.1, Windows 95, Windows 98, Windows 2000, or Windows NT. The software component represents common languages and functions conveniently present on this system to assist programs implementing the methods specific to this invention. Many high or low level computer languages can be used to program the analytic methods of this invention. Instructions can be interpreted during run-time or compiled. Preferred languages include C/ C++, FORTRAN and JAVA. Most preferably, the methods of this invention are programmed in mathematical software packages that allow symbolic entry of equations and high-level specification of processing, including some or all of the algorithms to be used, thereby freeing a user of the need to procedurally program individual equations or algorithms. Such packages include Mathlab from Mathworks (Natick, MA), Mathematica® from Wolfram Research (Champaign, IL), or S-Plus® from Math Soft (Cambridge, MA).

Specifically, the software component includes the analytic methods of the invention as programmed in a procedural language or symbolic package.

The software to be included with the kit comprises the data analysis methods of the invention as disclosed herein. In particular, the software may include mathematical routines for marker discovery, including the calculation of correlation coefficients between clinical categories (*i.e.*, ER status) and marker expression. The software may also include mathematical routines for calculating the correlation between sample marker expression and control marker expression, using array-generated fluorescence data, to determine the clinical classification of a sample.

In an exemplary implementation, to practice the methods of the present invention, a user first loads experimental data into the computer system. These data can be directly entered by the user from a monitor, keyboard, or from other computer systems linked by a network connection, or on removable storage media such as a CD-ROM, floppy disk (not illustrated), tape drive (not illustrated), ZIP® drive (not illustrated) or through the network. Next the user causes execution of expression profile analysis software which performs the methods of the present invention.

In another exemplary implementation, a user first loads experimental data and/or databases into the computer system. This data is loaded into the memory from the storage media or from a remote computer, preferably from a dynamic geneset database system, through the network. Next the user causes execution of software that performs the steps of the present invention.

Alternative computer systems and software for implementing the analytic methods of this invention will be apparent to one of skill in the art and are intended to be comprehended within the accompanying claims. In particular, the accompanying claims are intended to include the alternative program structures for implementing the methods of this invention that will be readily apparent to one of skill in the art.

6. EXAMPLES

Materials And Methods

117 tumor samples from breast cancer patients were collected. RNA samples were then prepared, and each RNA sample was profiled using inkjet-printed microarrays. Marker genes were then identified based on expression patterns; these genes were then used to train classifiers, which used these marker genes to classify tumors into diagnostic and prognostic categories. Finally, these marker genes were used to predict the diagnostic and prognostic outcome for a group of individuals..

1. Sample collection

117 breast cancer patients treated at The Netherlands Cancer Institute / Antoni van Leeuwenhoek Hospital, Amsterdam, The Netherlands, were selected on the basis of the following clinical criteria (data extracted from the medical records of the NKI/AvL Tumor Register, Biometrics Department).

Group 1 (n=97, 78 for training, 19 for independent tests) was selected on the basis of: (1) primary invasive breast carcinoma <5 cm (T1 or T2); (2) no axillary metastases (N0); (3) age at diagnosis <55 years; (4) calender year of diagnosis 1983-1996; and (5) no prior malignancies (excluding carcinoma in situ of the cervix or basal cell carcinoma of the skin). All patients were treated by modified radical mastectomy (n=34) or breast conserving treatment (n=64), including axillary lymph node dissection. Breast conserving treatment consisted of excision of the tumor, followed by radiation of the whole breast to a dosis of 50 Gy, followed by a boost varying from 15 to 25 Gy. Five patients received adjuvant systemic therapy consisting of chemotherapy (n=3) or hormonal therapy (n=2), all other patients did not receive additional treatment. All patients were followed at least annually for a period of at least 5 years. Patient follow-up information was extracted from the Tumor Registry of the Biometrics Department.

Group 2 (n=20) was selected as: (1) carriers of a germline mutation in *BRCA1* or *BRCA2*; and (2) having primary invasive breast carcinoma. No selection or exclusion was made based on tumor size, lymph node status, age at diagnosis, calender year of diagnosis, other malignancies. Germline mutation status was known prior to this research protocol.

Information about individual from which tumor samples were collected include: year of birth; sex; whether the individual is pre- or post-menopausal; the year of diagnosis; the number of positive lymph nodes and the total number of nodes; whether there was surgery, and if so, whether the surgery was breast-conserving or radical; whether there was radiotherapy, chemotherapy or hormonal therapy. The tumor was graded according to the formula P=TNM, where T is the tumor size (on a scale of 0-5); N is the number of nodes that are positive (on a scale of 0-4); and M is metastases (0 = absent, 1 = present). The tumor was also classified according to stage, tumor type (*in situ* or invasive; lobular or ductal; grade) and the presence or absence of the estrogen and progesterone receptors. The progression of the cancer was described by (where applicable): distant metastases; year of distant metastases, year of death, year of last follow-up; and *BRCA1* genotype.

2. Tumors:

Germline mutation testing of *BRCA1* and *BRCA2* on DNA isolated from peripheral blood lymphocytes includes mutation screening by a Protein Truncation Test (PTT) of exon 11 of *BRCA1* and exon 10 and 11 of *BRCA2*, deletion PCR of *BRCA1* genomic deletion of exon 13 and 22, as well Denaturing Gradient Gel Electrophoresis (DGGE) of the remaining exons. Aberrant bands were all confirmed by genomic sequencing analyzed on a ABI3700 automatic sequencer and confirmed on a independent DNA sample.

From all, tumor material was snap frozen in liquid nitrogen within one hour after surgery. Of the frozen tumor material an H&E (hematoxylin-eosin) stained section was prepared prior to and after cutting slides for RNA isolation. These H&E frozen sections were assessed for the percentage of tumor cells; only samples with >50% tumor cells were selected for further study.

For all tumors, surgical specimens fixed in formaldehyde and embedded in paraffin were evaluated according to standard histopathological procedures. H&E stained paraffin sections were examined to assess tumor type (e.g., ductal or lobular according to the WHO classification); to assess histologic grade according the method described by Elston and Ellis (grade 1-3); and to assess the presence of lymphangio-invasive growth and the presence of an extensive lymphocytic infiltrate. All histologic factors were independently assessed by two pathologists (MV and JL); consensus on differences was reached by examining the slides together. A representative slide of each tumor was used for immunohistochemical staining with antibodies directed against the estrogen- and progesterone receptor by standard procedures. The staining result was scored as the percentage of positively staining nuclei (0%, 10%, 20%, etc., up to 100%).

3. Amplification, labeling, and hybridization

The outline for the production of marker-derived nucleic acids and hybridization of the nucleic acids to a microarray are outlined in FIG. 2. 30 frozen sections of 30 µM thickness were used for total RNA isolation of each snap frozen tumor specimen. Total RNA was isolated with RNAzol™ B (Campro Scientific, Veenendaal, The Netherlands) according to the manufacturers protocol, including homogenization of the tissue using a Polytron PT-MR2100 (Merck, Amsterdam, The Netherlands) and finally dissolved in RNAse-free H_2O. The quality of the total RNA was assessed by A260/A280 ratio and had to be between 1.7 and 2.1 as well as visual inspection of the RNA on an agarose gel which should indicate a stronger 28S ribosomal RNA band compared to the 18S

ribosomal RNA band. subsequently, 25μg of total RNA was DNase treated using the Qiagen RNase-free DNase kit and RNeasy spin columns (Qiagen Inc, GmbH, Germany) according to the manufacturers protocol. DNase treated total RNA was dissolved in RNase-free H_2O to a final concentration of 0.2μg/μl.

5μg total RNA was used as input for cRNA synthesis. An oligo-dT primer containing a T7 RNA polymerase promoter sequence was used to prime first strand cDNA synthesis, and random primers (pdN6) were used to prime second strand cDNA synthesis by MMLV reverse transcriptase. This reaction yielded a double-stranded cDNA that contained the T7 RNA polymerase (T7RNAP) promoter. The double-stranded cDNA was then transcribed into cRNA by T7RNAP.

cRNA was labeled with Cy3 or Cy5 dyes using a two-step process. First, allylamine-derivitized nucleotides were enzymatically incorporated into cRNA products. For cRNA labeling, a 3:1 mixture of 5-(3-Aminoallyl)uridine 5'-triphosphate (Sigma) and UTP was substituted for UTP in the in vitro transcription (IVT) reaction. Allylamine-derivitized cRNA products were then reacted with N-hydroxy succinimide esters of Cy3 or Cy5 (CyDye, Amersham Pharmacia Biotech). 5μg Cy5-labeled cRNA from one breast cancer patient was mixed with the same amount of Cy3-labeled product from a pool of equal amount of cRNA from each individual sporadic patient.

Microarray hybridizations were done in duplicate with fluor reversals. Before hybridization, labeled cRNAs were fragmented to an average size of ~50-100nt by heating at 60 °C in the presence of 10 mM ZnCl2. Fragmented cRNAs were added to hybridization buffer containing 1 M NaCl, 0.5% sodium sarcosine and 50 mM MES, pH 6.5, which stringency was regulated by the addition of formamide to a final concentration of 30%. Hybridizations were carried out in a final volume of 3 mls at 40 °C on a rotating platform in a hybridization oven (Robbins Scientific) for 48h. After hybridization, slides were washed and scanned using a confocal laser scanner (Agilent Technologies). Fluorescence intensities on scanned images were quantified, normalized and corrected.

4. Pooling of samples

The reference cRNA pool was formed by pooling equal amount of cRNAs from each individual sporadic patient, for a total of 78 tumors.

5. 25k human microarray

Surface-bound oligonucleotides were synthesized essentially as proposed by Blanchard *et al., Biosens. Bioelectron.* 6(7):687-690 (1996); see also Hughes et al., *Nature*

Biotech. 19(4):342-347 (2000). Hydrophobic glass surfaces (3 inches by 3 inches) containing exposed hydroxyl groups were used as substrates for nucleotide synthesis. Phosphoramidite monomers were delivered to computer-defined positions on the glass surfaces using ink-jet printer heads. Unreacted monomers were then washed away and the ends of the extended oligonucleotides were deprotected. This cycle of monomer coupling, washing and deprotection was repeated for each desired layer of nucleotide synthesis. Oligonucleotide sequences to be printed were specified by computer files.

Microarrays containing approximately 25,000 human gene sequences (Hu25K microarrays) were used for this study. Sequences for microarrays were selected from RefSeq (a collection of non-redundant mRNA sequences, located on the Internet at nlm.nih.gov/LocusLink/refseq.html) and Phil Green EST contigs, which is a collection of EST contigs assembled by Dr. Phil Green et al at the University of Washington (Ewing and Green, Nat. Genet. 25(2):232-4 (2000)), available on the Internet at phrap.org/est_assembly/index.html. Each mRNA or EST contig was represented on Hu25K microarray by a single 60mer oligonucleotide essentially as described in Hughes *et al., Nature Biotech.* 19(4):342-347 and in International Publication WO 01/06013, published January 25, 2001, and in International Publication WO 01/05935, published January 25, 2001, except that the rules for oligo screening were modified to remove oligonucleotides with more than 30%C or with 6 or more contiguous C residues.

Example 1: Differentially regulated gene sets and overall expression patterns of breast cancer tumors

Of the approximately 25,000 sequences represented on the microarray, a group of approximately 5,000 genes that were significantly regulated across the group of samples was selected. A gene was determined to be significantly differentially regulated with cancer of the breast if it showed more than two-fold of transcript changes as compared to a sporadic tumor pool, and if the p-value for differential regulation (Hughes *et al., Cell* 102:109-126 (2000)) was less than 0.01 either upwards or downwards in at least five out of 98 tumor samples.

An unsupervised clustering algorithm allowed us to cluster patients based on their similarities measured over this set of ~5,000 significant genes. The similarity measure between two patients x and y is defined as

$$S = 1 - \left[\sum_{i=1}^{N_V} \frac{(x_i - \bar{x})}{\sigma_{x_i}} \frac{(y_i - \bar{y})}{\sigma_{y_i}} \Bigg/ \sqrt{\sum_{i=1}^{N_V} \left(\frac{x_i - \bar{x}}{\sigma_{x_i}} \right)^2 \sum_{i=1}^{N_V} \left(\frac{y_i - \bar{y}}{\sigma_{y_i}} \right)^2} \right] \qquad \text{Equation (5)}$$

In Equation (5), x and y are two patients with components of log ratio x_i and y_i, i= 1,..., N=5,100. Associated with every value x_i is error σ_{x_i}. The smaller the value σ_{x_i}, the more reliable the measurement x_i. $\bar{x} = \sum_{i=1}^{N_V} \frac{x_i}{\sigma_{x_i}^2} \Big/ \sum_{i=1}^{N_V} \frac{1}{\sigma_{x_i}^2}$ is the error-weighted arithmetic mean.

The use of correlation as similarity metric emphasizes the importance of co-regulation in clustering rather than the amplitude of regulations.

The set of approximately 5,000 genes can be clustered based on their similarities measured over the group of 98 tumor samples. The similarity measure between two genes was defined in the same way as in Equation (1) except that now for each gene, there are 98 components of log ratio measurements.

The result of such a two-dimensional clustering is displayed in FIG 3. Two distinctive patterns emerge from the clustering. The first pattern consists of a group of patients in the lower part of the plot whose regulations are very different from the sporadic pool. The other pattern is made of a group of patients in the upper part of the plot whose expressions are only moderately regulated in comparison with the sporadic pool. These dominant patterns suggest that the tumors can be unambiguously divided into two distinct types based on this set of ~5,000 significant genes.

To help understand these patterns, they were associated with estrogen-receptor (ER), proestrogen receptor (PR), tumor grade, presence of lymphocytic infiltrate, and angioinvasion (FIG. 3). The lower group in FIG 3, which features the dominant pattern, consists of 36 patients. Of the 39 ER-negative patients, 34 patients are clustered together in this group. From FIG. 4, it was observed that the expression of estrogen receptor alpha gene *ESR1* and a large group of co-regulated genes are consistent with this expression pattern.

From FIG. 3 and FIG. 4, it was concluded that gene expression patterns can be used to classify tumor samples into subgroups of diagnostic interest. Thus, genes co-

regulated across 98 tumor samples contain information about the molecular basis of breast cancers. The combination of clinical data and microarray measured gene abundance of *ESR1* demonstrates that the distinct types are related to, or at least are reported by, the ER status.

Example 2: Identification of Genetic Markers Distinguishing Estrogen Receptor (+) From Estrogen Receptor (-) Patients

The results described in this Example allow the identification of expression marker genes that differentiate two major types of tumor cells: "ER-negative" group and "ER-positive" group. The differentiation of samples by ER(+) status was accomplished in three steps: (1) identification of a set of candidate marker genes that correlate with ER level; (2) rank-ordering these candidate genes by strength of correlation; (3) optimization of the number of marker genes; and (4) classifying samples based on these marker genes.

1. Selection of candidate discriminating genes

In the first step, a set of candidate discriminating genes was identified based on gene expression data of training samples. Specifically, we calculated the correlation coefficients ρ between the category numbers or ER level and logarithmic expression ratio $\vec{r}$ across all the samples for each individual gene:

$$\rho = (\vec{c} \bullet \vec{r}) / (\|\vec{c}\| \cdot \|\vec{r}\|) \qquad \text{Equation (2)}$$

The histogram of resultant correlation coefficients is shown in FIG. 5A as a gray line. While the amplitude of correlation or anti-correlation is small for the majority of genes, the amplitude for some genes is as great as 0.5. Genes whose expression ratios either correlate or anti-correlate well with the diagnostic category of interest are used as reporter genes for the category.

Genes having a correlation coefficient larger than 0.3 ("correlated genes") or less than –0.3 ("anti-correlated genes") were selected as reporter genes. The threshold of 0.3 was selected based on the correlation distribution for cases where there is no real correlation (one can use permutations to determine this distribution). Statistically, this distribution width depends upon the number of samples used in the correlation calculation. The distribution width for control cases (no real correlation) is approximately $1/\sqrt{n-3}$,

where n = the number of samples. In our case, n = 98. Therefore, a threshold of 0.3 roughly corresponds to 3 - σ in the distribution (3 X $1/\sqrt{n-3}$).

2,460 such genes were found to satisfy this criterion. In order to evaluate the significance of the correlation coefficient of each gene with the ER level, a bootstrap technique was used to generate Monte-Carlo data that randomize the association between gene expression data of the samples and their categories. The distribution of correlation coefficients obtained from one Monte-Carlo trial is shown as a dashed line in FIG 5A. To estimate the significance of the 2,460 marker genes as a group, 10,000 Monte-Carlo runs were generated. The collection of 10,000 such Monte-Carlo trials forms the null hypothesis. The number of genes that satisfy the same criterion for Monte-Carlo data varies from run to run. The frequency distribution from 10,000 Monte-Carlo runs of the number of genes having correlation coefficients of >0.3 or <-0.3 is displayed in FIG. 5B. Both the mean and maximum value are much smaller than 2,460. Therefore, the significance of this gene group as the discriminating gene set between ER(+) and ER(-) samples is estimated to be greater than 99.99%.

2. Rank-ordering of candidate discriminating genes

In the second step, genes on the candidate list were rank-ordered based on the significance of each gene as a discriminating gene. The markers were rank-ordered either by amplitude of correlation, or by using a metric similar to a Fisher statistic:

$$t = \left(\langle x_1\rangle - \langle x_2\rangle\right) \Big/ \sqrt{\left[\sigma_1^2(n_1-1)+\sigma_2^2(n_2-1)\right]/(n_1+n_2-1)/(1/n_1+1/n_2)} \qquad \text{Equation (3)}$$

In Equation (3), $\langle x_1\rangle$ is the error-weighted average of log ratio within the ER(-), and $\langle x_2\rangle$ is the error-weighted average of log ratio within the ER(+) group. σ_1 is the variance of log ratio within the ER(-) group and n_1 is the number of samples that had valid measurements of log ratios. σ_2 is the variance of log ratio within the ER(+) group and n_2 is the number of samples that had valid measurements of log ratios. The *t*-value in Equation (3) represents the variance-compensated difference between two means. The confidence level of each gene in the candidate list was estimated with respect to a null hypothesis derived from the

actual data set using a bootstrap technique; that is, many artificial data sets were generated by randomizing the association between the clinical data and the gene expression data.

3. Optimization of the number of marker genes

The leave-one-out method was used for cross validation in order to optimize the discriminating genes. For a set of marker genes from the rank-ordered candidate list, a classifier was trained with 97 samples, and was used to predict the status of the remaining sample. The procedure was repeated for each of the samples in the pool, and the number of cases where the prediction for the one left out is wrong or correct was counted.

The above performance evaluation from leave-one-out cross validation was repeated by successively adding more marker genes from the candidate list. The performance as a function of the number of marker genes is shown in FIG. 6. The error rates for type 1 and type 2 errors varied with the number of marker genes used, but were both minimal while the number of the marker genes is around 550. Therefore, we consider this set of 550 genes is considered the optimal set of marker genes that can be used to classify breast cancer tumors into "ER-negative" group and "ER-positive" group. FIG. 7 shows the classification of patients as ER(+) or ER(-) based on this 550 marker set. FIG. 8 shows the correlation of each tumor to the ER-negative template verse the correlation of each tumor to the ER-positive template.

4. Classification based on marker genes

In the third step, a set of classifier parameters was calculated for each type of training data set based on either of the above ranking methods. A template for the ER(-) group ($\vec{z}_1$) was generated using the error-weighted log ratio average of the selected group of genes. Similarly, a template for ER(+) group (called $\vec{z}_2$) was generated using the error-weighted log ratio average of the selected group of genes. Two classifier parameters (P_1 and P_2) were defined based on either correlation or distance. P_1 measures the similarity between one sample $\vec{y}$ and the ER(-) template $\vec{z}_1$ over this selected group of genes. P_2 measures the similarity between one sample $\vec{y}$ and the ER(+) template $\vec{z}_2$ over this selected group of genes. The correlation P_i is defined as:

$$P_i = (\vec{z}_i \bullet \vec{y}) / (\|\vec{z}_i\| \cdot \|\vec{y}\|) \qquad \text{Equation} \quad (1)$$

A "leave-one-out" method was used to cross-validate the classifier built based on the marker genes. In this method, one sample was reserved for cross validation each time the classifier was trained. For the set of 550 optimal marker genes, the classifier was trained with 97 of the 98 samples, and the status of the remaining sample was predicted. This procedure was performed with each of the 98 patients. The number of cases where the prediction was wrong or correct was counted. It was further determined that subsets of as few as ~50 of the 2,460 genes are able classify tumors as ER(+) or ER(-) nearly as well as using the total set.

In a small number of cases, there was disagreement between classification by the 550 marker set and a clinical classification. In comparing the microarray measured log ratio of expression for *ESR1* to the clinical binary decision (negative or positive) of ER status for each patient, it was seen that the measured expression is consistent with the qualitative category of clinical measurements (mixture of two methods) for the majority of tumors. For example, two patients who were clinically diagnosed as ER(+) actually exhibited low expression of *ESR1* from microarray measurements and were classified as ER negative by 550 marker genes. Additionally, 3 patients who were clinically diagnosed as ER(-) exhibited high expression of *ESR1* from microarray measurements and were classified as ER(+) by the same 550 marker genes. Statistically, however, microarray measured gene expression of *ESR*1 correlates with the dominant pattens better than clinically determined ER status.

Example 3: Identification of Genetic Markers Distinguishing *BRCA1* Tumors From Sporadic Tumors in Estrogen Receptor (-) Patients

The *BRCA1* mutation is one of the major clinical categories in breast cancer tumors. It was determined that of tumors of 38 patients in the ER(-) group, 17 exhibited the *BRCA1* mutation, while 21 were sporadic tumors. A method was therefore developed that enabled the differentiation of the 17 *BRCA1* mutation tumors from the 21 sporadic tumors in the ER(-) group.

1. Selection of candidate discriminating genes

In the first step, a set of candidate genes was identified based on the gene expression patterns of these 38 samples. We first calculated the correlation between the

BRCA1-mutation category number and the expression ratio across all 38 samples for each individual gene by Equation (2). The distribution of the correlation coefficients is shown as a histogram defined by the solid line in FIG. 9A. We observed that, while the majority of genes do not correlate with *BRCA1* mutation status, a small group of genes correlated at significant levels. It is likely that genes with larger correlation coefficients would serve as reporters for discriminating tumors of *BRCA1* mutation carriers from sporadic tumors within the ER(-) group.

In order to evaluate the significance of each correlation coefficient with respect to a null hypothesis that such correlation coefficient could be found by chance, a bootstrap technique was used to generate Monte-Carlo data that randomizes the association between gene expression data of the samples and their categories. 10,000 such Monte-Carlo runs were generated as a control in order to estimate the significance of the marker genes as a group. A threshold of 0.35 in the absolute amplitude of correlation coefficients (either correlation or anti-correlation) was applied both to the real data and the Monte-Carlo data. Following this method, 430 genes were found to satisfy this criterion for the experimental data. The p-value of the significance, as measured against the 10,000 Monte-Carlo trials, is approximately 0.0048 (FIG. 9B). That is, the probability that this set of 430 genes contained useful information about BRCA1-like tumors vs sporadic tumors exceeds 99%.

2. Rank-ordering of candidate discriminating genes

In the second step, genes on the candidate list were rank-ordered based on the significance of each gene as a discriminating gene. Here, we used the absolute amplitude of correlation coefficients to rank order the marker genes.

3 Optimization of discriminating genes

In the third step, a subset of genes from the top of this rank-ordered list was used for classification. We defined a *BRCA1* group template (called $\vec{z}_1$) by using the error-weighted log ratio average of the selected group of genes. Similarly, we defined a non-*BRCA1* group template (called $\vec{z}_2$) by using the error-weighted log ratio average of the selected group of genes. Two classifier parameters (P1 and P2) were defined based on either correlation or distance. P1 measures the similarity between one sample $\vec{y}$ and the *BRCA1* template $\vec{z}_1$ over this selected group of genes. P2 measures the similarity between

one sample $\vec{y}$ and the non-*BRCA1* template $\vec{z}_2$ over this selected group of genes. For correlation, P1 and P2 were defined in the same way as in Equation (4).

The leave-one-out method was used for cross validation in order to optimize the discriminating genes as described in Example 2. For a set of marker genes from the rank-ordered candidate list, the classifier was trained with 37 samples the remaining one was predicted. The procedure was repeated for all the samples in the pool, and the number of cases where the prediction for the one left out is wrong or correct was counted.

To determine the number of markers constituting a viable subset, the above performance evaluation from leave-one-out cross validation was repeated by cumulatively adding more marker genes from the candidate list. The performance as a function of the number of marker genes is shown in FIG. 10. The error rates for type 1 (false negative) and type 2 (false positive) errors (Bendat & Piersol, RANDOM DATA ANALYSIS AND MEASUREMENT PROCEDURES, 2D ED., Wiley Interscience, p. 89) reached optimal ranges when the number of the marker genes is approximately 100. Therefore, a set of about 100 genes is considered to be the optimal set of marker genes that can be used to classify tumors in the ER(-) group as either *BRCA1*-related tumors or sporadic tumors.

The classification results using the optimal 100 genes are shown in FIGS. 11A and 11B. As shown in Figure 11A, the co-regulation patterns of the sporadic patients differ from those of the *BRCA1* patients primarily in the amplitude of regulation. Only one sporadic tumor was classified into the *BRCA1* group. Patients in the sporadic group are not necessarily *BRCA1* mutation negative; however, it is estimated that only approximately 5% of sporadic tumors are indeed *BRCA1*-mutation carriers.

Example 4: Identification of Genetic Markers Distinguishing Sporadic Tumor Patients with >5 Year Versus <5 Year Survival Times

78 tumors from sporadic breast cancer patients were used to explore prognostic predictors from gene expression data. Of the 78 samples in this sporadic breast cancer group, 44 samples were known clinically to have had no distant metastases within 5 years since the initial diagnosis ("no distant metastases group") and 34 samples had distant metastases within 5 years since the initial diagnosis ("distant metastases group"). A group of 231 markers, and optimally a group of 70 markers, was identified that allowed differentiation between these two groups.

1. Selection of candidate discriminating genes

In the first step, a set of candidate discriminating genes was identified based on gene expression data of these 78 samples. The correlation between the prognostic category number (distant metastases vs no distant metastases) and the logarithmic expression ratio across all samples for each individual gene was calculated using Equation (2). The distribution of the correlation coefficients is shown as a solid line in FIG. 12A. FIG. 12A also shows the result of one Monte-Carlo run as a dashed line. We observe that even though the majority of genes do not correlate with the prognostic categories, a small group of genes do correlate. It is likely that genes with larger correlation coefficients would be more useful as reporters for the prognosis of interest – distant metastases group and no distant metastases group.

In order to evaluate the significance of each correlation coefficient with respect to a null hypothesis that such correlation coefficient can be found by chance, we used a bootstrap technique to generate data from 10,000 Monte-Carlo runs as a control (FIG. 12B). We then selected genes that either have the correlation coefficient larger than 0.3 ("correlated genes") or less than –0.3 ("anti-correlated genes"). The same selection criterion was applied both to the real data and the Monte-Carlo data. Using this comparison, 231 markers from the experimental data were identified that satisfy this criterion. The probability of this gene set for discriminating patients between the distant metastases group and the no distant metastases group being chosen by random fluctuation is approximately 0.003.

2. Rank-ordering of candidate discriminating genes

In the second step, genes on the candidate list were rank-ordered based on the significance of each gene as a discriminating gene. Specifically, a metric similar to a "Fisher" statistic, defined in Equation (3), was used for the purpose of rank ordering. The confidence level of each gene in the candidate list was estimated with respect to a null hypothesis derived from the actual data set using the bootstrap technique. Genes in the candidate list can also be ranked by the amplitude of correlation coefficients.

3. Optimization of discriminating genes

In the third step, a subset of 5 genes from the top of this rank-ordered list was selected to use as discriminating genes to classify 78 tumors into a "distant metastases group" or a "no distant metastases group". The leave-one-out method was used for cross validation. Specifically, 77 samples defined a classifier based on the set of selected

discriminating genes, and these were used to predict the remaining sample. This procedure was repeated so that each of the 78 samples was predicted. The number of cases in which predictions were correct or incorrect were counted. The performance of the classifier was measured by the error rates of type 1 and type 2 for this selected gene set.

We repeated the above performance evaluation procedure, adding 5 more marker genes each time from the top of the candidate list, until all 231 genes were used. As shown in FIG. 13, the number of mis-predictions of type 1 and type 2 errors change dramatically with the number of marker genes employed. The combined error rate reached a minimum when 70 marker genes from the top of our candidate list never used. Therefore, this set of 70 genes is the optimal, preferred set of marker genes useful for the classification of sporadic tumor patients into either the distant metastases or no distant metastases group. Fewer or more markers also act as predictors, but are less efficient, either because of higher error rates, or the introduction of statistical noise.

4. Reoccurrence probability curves

The prognostic classification of 78 patients with sporadic breast cancer tumors into two distinct subgroups was predicted based on their expression of the 70 optimal marker genes (FIGS. 14 and 15).

To evaluate the prognostic classification of sporadic patients, we predicted the outcome of each patient by a classifier trained by the remaining 77 patients based on the 70 optimal marker genes. FIG. 16 plots the distant metastases probability as a function of the time since initial diagnosis for the two predicted groups. The difference between these two reoccurrence curves is significant. Using the χ^2 test (S-PLUS 2000 Guide to Statistics, vol. 2, MathSoft, p. 44), the p-value is estimated to be $\sim 10^{-9}$. The distant metastases probability as a function of the time since initial diagnosis was also compared between ER(+) and ER(-) individuals (FIG. 17), PR(+) and PR(-) individuals (FIG. 18), and between individuals with different tumor grades (FIGS. 19A, 19B). In comparison, the p-values for the differences between two prognostic groups based on clinical data are much less significant than that based on gene expression data, ranging from 10^{-3} to 1.

To parameterize the reoccurrence probability as a function of time since initial diagnosis, the curve was fitted to one type of survival model – "normal":

$$P = \alpha \times \exp\left(-t^2/\tau^2\right) \qquad (4)$$

For fixed $\alpha = 1$, we found that $\tau = 125$months for patients in the no distant metastases group and $\tau = 36$ months for patients in the distant metastases group. Using tumor grades, we

found $\tau = 100$ months for patients with tumor grades 1 and 2 and $\tau = 60$ for patients with tumor grade 3. It is accepted clinical practice that tumor grades are the best available prognostic predictor. However, the difference between the two prognostic groups classified based on 70 marker genes is much more significant than those classified by the best available clinical information.

5. Prognostic prediction for 19 independent sporadic tumors

To confirm the proposed prognostic classification method and to ensure the reproducibility, robustness, and predicting power of the 70 optimal prognostic marker genes, we applied the same classifier to 19 independent tumor samples from sporadic breast cancer patients, prepared separately at The Netherlands Cancer Institute (NKI). The same reference pool was used.

The classification results of 19 independent sporadic tumors are shown in Figure 20. FIG. 20A shows the log ratio of expression regulation of the same 70 optimum marker genes. Based on our classifier model, we expected the misclassification of 19*(6+7)/78 = 3.2 tumors. Consistently, (1+3) = 4 of 19 tumors were misclassified.

6. Clinical parameters as a group vs. microarray data – Results of logistic regression

In the previous section, the predictive power of each individual clinical parameter was compared with that of the expression data. However, it is more meaningful to combine all the clinical parameters as a group, and then compare them to the expression data. This requires multi-variant modeling; the method chosen was logistic regression. Such an approach also demonstrates how much improvement the microarray approach adds to the results of the clinical data.

The clinical parameters used for the multi-variant modeling were: (1) tumor grade; (2) ER status; (3) presence or absence of the progestogen receptor (PR); (4) tumor size; (5) patient age; and (6) presence or absence of angioinvasion. For the microarray data, two correlation coefficients were used. One is the correlation to the mean of the good prognosis group (C1) and the other is the correlation to the mean of the bad prognosis group (C2). When calculating the correlation coefficients for a given patient, this patient is excluded from either of the two means.

The logistic regression optimizes the coefficient of each input parameter to best predict the outcome of each patient. One way to judge the predictive power of each input parameter is by how much deviance (similar to Chi-square in the linear regression, see

for example, Hasomer & Lemeshow, APPLIED LOGISTIC REGRESSION, John Wiley & Sons, (2000)) the parameter accounts for. The best predictor should account for most of the deviance. To fairly assess the predictive power, each parameter was modeled independently. The microarray parameters explain most of the deviance, and hence are powerful predictors.

The clinical parameters, and the two microarray parameters, were then monitored as a group. The total deviance explained by the six clinical parameters was 31.5, and total deviance explained by the microarray parameters was 39.4. However, when the clinical data was modeled first, and the two microarray parameters added, the final deviance accounted for is 57.0.

The logistic regression computes the likelihood that a patient belongs to the good or poor prognostic group. FIGS. 21A and 21B show the sensitivity vs. (1-specificity). The plots were generated by varying the threshold on the model predicted likelihood. The curve which goes through the top left corner is the best (high sensitivity with high specificity). The microarray outperformed the clinical data by a large margin. For example, at a fixed sensitivity of around 80%, the specificity was ~80% from the microarray data, and ~65% from the clinical data for the good prognosis group. For the poor prognosis group, the corresponding specificities were ~80% and ~70%, again at a fixed sensitivity of 80%. Combining the microarray data with the clinical data further improved the results. The result can also be displayed as the total error rate as the function of the threshold in FIG. 21C. At all possible thresholds, the error rate from the microarray was always smaller than that from the clinical data. By adding the microarray data to the clinical data, the error rate is further reduced, as one can see in Figure 21C.

Odds ratio tables can be created from the prediction of the logistic regression. The probability of a patient being in the good prognosis group is calculated by the logistic regression based on different combinations of input parameters (clinical and/or microarray). Patients are divided into the following four groups according to the prediction and the true outcome: (1) predicted good and truly good, (2) predicted good but truly poor, (3) predicted poor but truly good, (4) predicted poor and truly poor. Groups (1) & (4) represent correct predictions, while groups (2) & (3) represent mis-predictions. The division for the prediction is set at probability of 50%, although other thresholds can be used. The results are listed in Table 7. It is clear from Table 7 that microarray profiling (Table 7.3 & 7.10) outperforms any single clinical data (Table 7.4-7.9) and the combination of the clinical data (Table 7.2). Adding the micro-array profiling in addition to the clinical data give the best results (Table 7.1).

For microarray profiling, one can also make a similar table (Table 7.11) without using logistic regression. In this case, the prediction was simply based on C1-C2 (greater than 0 means good prognosis, less than 0 mean bad prognosis).

Table 7.1 Prediction by clinical+microarray		
	Predicted good	Predicted poor
true good	39	5
true poor	4	30

Table 7.2 Prediction by clinical alone		
	Predicted good	Predicted poor
true good	34	10
true poor	12	22

Table 7.3 Prediction by microarray		
	predicted good	Predicted poor
true good	39	5
true poor	10	24

Table 7.4 Prediction by grade		
	Predicted good	Predicted poor
true good	23	21
true poor	5	29

Table 7.5 Prediction by ER		
	Predicted good	Predicted poor
true good	35	9
true poor	21	13

Table 7.6 Prediction by PR		
	Predicted good	Predicted poor
true good	35	9
true poor	18	16

Table 7.7 Prediction by size		
	Predicted good	Predicted poor
true good	35	9
true poor	13	21

Table 7.8 Prediction by age		
	Predicted good	Predicted poor
true good	33	11
true poor	15	19

Table 7.9 Prediction by angioinvasion		
	Predicted good	Predicted poor
true good	37	7
true poor	19	15

Table 7.10 Prediction by dC (C1-C2)		
	Predicted good	Predicted poor
true good	36	8
true poor	6	28

Table 7.11 No logistic regression, simply judged by C1-C2		
	Predicted good	Predicted poor
true good	37	7
true poor	6	28

Example 5. Concept of mini-array for diagnosis purposes.

All genes on the marker gene list for the purpose of diagnosis and prognosis can be synthesized on a small-scale microarray using ink-jet technology. A microarray with genes for diagnosis and prognosis can respectively or collectively be made. Each gene on the list is represented by single or multiple oligonucleotide probes, depending on its sequence uniqueness across the genome. This custom designed mini-array, in combination with sample preparation protocol, can be used as a diagnostic/prognostic kit in clinics.

Example 6. Biological Significance of diagnostic marker genes

The public domain was searched for the available functional annotations for the 430 marker genes for *BRCA1* diagnosis in Table 3. The 430 diagnostic genes in Table 3 can be divided into two groups: (1) 196 genes whose expressions are highly expressed in *BRCA1*-like group; and (2) 234 genes whose expression are highly expressed sporadic group. Of the 196 *BRCA1* group genes, 94 are annotated. Of the 234 sporadic group genes, 100 are annotated. The terms "T-cell", " B-cell" or "immunoglobulin" are involved in 13 of the 94 annotated genes, and in 1 of the 100 annotated genes, respectively. Of 24,479 genes represented on the microarrays, there are 7,586 genes with annotations to date. "T-cell", B-cell" and "immunoglobulin" are found in 207 of these 7,586 genes. Given this, the p-value of the 13 "T-cell", "B-cell" or "immunoglobulin" genes in the *BRCA1* group is very significant (p-value = 1.1x10-6). In comparison, the observation of 1 gene relating to "T-cell", "B-cell", or "immunoglobulin" in the sporadic group is not significant (p-value = 0.18).

The observation that *BRCA1* patients have highly expressed lymphocyte (T-cell and B-cell) genes agrees with what has been seen from pathology that *BRCA1* breast tumor has more frequently associated with high lymphocytic infiltration than sporadic cases (Chappuis *et al.*, 2000, *Semin Surg Oncol* 18:287-295).

Example 7. Biological significance of prognosis marker genes

A search was performed for available functional annotations for the 231 prognosis marker genes (Table 5). The markers fall into two groups: (1) 156 markers

whose expressions are highly expressed in poor prognostic group; and (2) 75 genes whose expression are highly expressed in good prognostic group. Of the 156 markers, 72 genes are annotated; of the 75 genes, 28 genes are annotated.

Twelve of the 72 markers, but none of the 28 markers, are, or are associated with, kinases. In contrast, of the 7,586 genes on the microarray having annotations to date, only 471 involve kinases. On this basis, the p-value that twelve kinase-related markers in the poor prognostic group is significant (p-value = 0.001). Kinases are important regulators of intracellular signal transduction pathways mediating cell proliferation, differentiation and apoptosis. Their activity is normally tightly controlled and regulated. Overexpression of certain kinases is well known involving in oncogenesis, such as vascular endothelial growth factor receptor1 (VEGFR1 or FLT1), a tyrosine kinase in the poor prognosis group, which plays a very important role in tumor angiogenesis. Interestingly, vascular endothelial growth factor (VEGF), VEGFR's ligand, is also found in the prognosis group, which means both ligand and receptor are upregulated in poor prognostic individuals by an unknown mechanism.

Likewise, 16 of the 72 markers, and only two of the 28 markers, are, or are associated with, ATP-binding or GTP-binding proteins. In contrast, of the 7,586 genes on the microarray having annotations to date, only 714 and 153 involve ATP-binding and GTP-binding, respectively. On this basis, the p-value that 16 GTP- or ATP-binding-related markers in the poor prognosis group is significant (p-value 0.001 and 0.0038). Thus, the kinase- and ATP- or GTP-binding-related markers within the 72 markers can be used as prognostic indicators.

Cancer is characterized by deregulated cell proliferation. On the simplest level, this requires division of the cell or mitosis. By keyword searching, we found "cell division" or "mitosis" included in the annotations of 7 genes respectively in the 72 annotated markers from the 156 poor prognosis markers, but in none for the 28 annotated genes from 75 good prognosis markers. Of the 7,586 microarray markers with annotations, "cell division" is found in 62 annotations and "mitosis" is found in 37 annotations. Based on these findings, the p-value that seven cell division- or mitosis-related markers are found in the poor prognosis group is estimated to be highly significant (p-value = 3.5×10^{-5}). In comparison, the absence of cell division- or mitosis-related markers in the good prognosis group is not significant (p-value = 0.69). Thus, the seven cell division- or mitosis-related markers may be used as markers for poor prognosis.

Example 8: Construction of an artificial reference pool.

The reference pool for expression profiling in the above Examples was made by using equal amount of cRNAs from each individual patient in the sporadic group. In order to have a reliable, easy-to-made, and large amount of reference pool, a reference pool for breast cancer diagnosis and prognosis can be constructed using synthetic nucleic acid representing, or derived from, each marker gene. Expression of marker genes for individual patient sample is monitored only against the reference pool, not a pool derived from other patients.

To make the reference pool, 60-mer oligonucleotides are synthesized according to 60-mer ink-jet array probe sequence for each diagnostic/prognostic reporter genes, then double-stranded and cloned into pBluescript SK- vector (Stratagene, La Jolla, CA), adjacent to the T7 promoter sequence. Individual clones are isolated, and the sequences of their inserts are verified by DNA sequencing. To generate synthetic RNAs, clones are linearized with *Eco*RI and a T7 *in vitro* transcription (IVT) reaction is performed according to the MegaScript kit (Ambion, Austin, TX). IVT is followed by DNase treatment of the product. Synthetic RNAs are purified on RNeasy columns (Qiagen, Valencia, CA). These synthetic RNAs are transcribed, amplified, labeled, and mixed together to make the reference pool. The abundance of those synthetic RNAs are adjusted to approximate the abundance of the corresponding marker-derived transcripts in the real tumor pool.

Example 9: Use of single-channel data and a sample pol represented by stored values.

1. Creation of a reference pool of stored values ("mathematical sample pool")

The use of ratio-based data used in Examples 1-7, above, requires a physical reference sample. In the above Examples, a pool of sporadic tumor sample was used as the reference. Use of such a reference, while enabling robust prognostic and diagnostic predictions, can be problematic because the pool is typically a limited resource. A classifier method was therefore developed that does not require a physical sample pool, making application of this predictive and diagnostic technique much simpler in clinical applications.

To test whether single-channel data could be used, the following procedure was developed. First, the single channel intensity data for the 70 optimal genes, described in Example 4, from the 78 sporadic training samples, described in the Materials and Methods, was selected from the sporadic sample vs. tumor pool hybridization data. The 78 samples consisted of 44 samples from patients having a good prognosis and 34 samples from patients having a poor prognosis. Next, the hybridization intensities for these samples

were normalized by dividing by the median intensity of all the biological spots on the same microarray. Where multiple microarrays per sample were used, the average was taken across all of the microarrays. A log transform was performed on the intensity data for each of the 70 genes, or for the average intensity for each of the 70 genes where more than one microarray is hybridized, and a mean log intensity for each gene across the 78 sporadic samples was calculated. For each sample, the mean log intensities thus calculated were subtracted from the individual sample log intensity. This figure, the mean subtracted log(intensity) was then treated as the two color log(ratio) for the classifier by substitution into Equation (5). For new samples, the mean log intensity is subtracted in the same manner as noted above, and a mean subtracted log(intensity) calculated.

The creation of a set of mean log intensities for each gene hybridized creates a "mathematical sample pool" that replaces the quantity-limited "material sample pool." This mathematical sample pool can then be applied to any sample, including samples in hand and ones to be collected in the future. This "mathematical sample pool" can be updated as more samples become available.

2. Results

To demonstrate that the mathematical sample pool performs a function equivalent to the sample reference pool, the mean-subtracted-log(intensity) (single channel data, relative to the mathematical pool) vs. the log(ratio) (hybridizations, relative to the sample pool) was plotted for the 70 optimal reporter genes across the 78 sporadic samples, as shown in FIG. 22. The ratio and single-channel quantities are highly correlated, indicating both have the capability to report relative changes in gene expression. A classifier was then constructed using the mean-subtracted-log(intensity) following exactly the same procedure as was followed using the ratio data, as in Example 4.

As shown in FIGS. 23A and 23B, single-channel data was successful at classifying samples based on gene expression patterns. FIG. 23A shows samples grouped according to prognosis using single-channel hybridization data. The white line separates samples from patients classified as having poor prognoses (below) and good prognoses (above). FIG. 23B plots each sample as its expression data correlates with the good (open circles) or poor (filled squares) prognosis classifier parameter. Using the "leave-one-out" cross validation method, the classifier predicted 10 false positives out of 44 samples from patients having a good prognosis, and 6 false negatives out of 34 samples from patients having a poor prognosis, where a poor prognosis is considered a "positive." This outcome

is comparable to the use of the ratio-based classifier, which predicted 7 out of 44, and 6 out of 34, respectively.

In clinical applications, it is greatly preferable to have few false positives, which results in fewer under-treated patients. To conform the results to this preference, a classifier was constructed by ranking the patient sample according to its coefficient of correlation to the "good prognosis" template, and chose a threshold for this correlation coefficient to allow approximately 10% false negatives, *i.e.*, classification of a sample from a patient with poor prognosis as one from a patient with a good prognosis. Out of the 34 poor prognosis samples used herein, this represents a tolerance of 3 out of 34 poor prognosis patients classified incorrectly. This tolerance limit corresponds to a threshold 0.2727 coefficient of correlation to the "good prognosis" template. Results using this threshold are shown in FIGS. 24A and 24B. FIG. 24A shows single-channel hybridization data for samples ranked according to the coefficients of correlation with the good prognosis classifier; samples classified as "good prognosis" lie above the white line, and those classified as "poor prognosis" lie below. FIG. 24B shows a scatterplot of sample correlation coefficients, with three incorrectly classified samples lying to the right of the threshold correlation coefficient value. Using this threshold, the classifier had a false positive rate of 15 out of the 44 good prognosis samples. This result is not very different compared to the error rate of 12 out of 44 for the ratio based classifier.

In summary, the 70 reporter genes carry robust information about prognosis; the single channel data can predict the tumor outcome almost as well as the ratio based data, while being more convenient in a clinical setting.

7. REFERENCES CITED

All references cited herein are incorporated herein by reference in their entirety and for all purposes to the same extent as if each individual publication or patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

Many modifications and variations of the present invention can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. The specific embodiments described herein are offered by way of example only, and the invention is to be limited only by the terms of the appended claims along with the full scope of equivalents to which such claims are entitled.

What is claimed is:

1. A method for classifying a cell sample as ER(+) or ER(-) comprising detecting a difference in the expression by said cell sample of a first plurality of genes relative to a control, said first plurality of genes consisting of at least 5 of the genes corresponding to the markers listed in Table 1.

2. The method of claim 1, wherein said plurality consists of at least 50 of the genes corresponding to the markers listed in Table 1.

3. The method of claim 1, wherein said plurality consists of at least 100 of the genes corresponding to the markers listed in Table 1.

4. The method of claim 1, wherein said plurality consists of at least 200 of the genes corresponding to the markers listed in Table 1.

5. The method of claim 1, wherein said plurality consists of at least 500 of the genes corresponding to the markers listed in Table 1.

6. The method of claim 1, wherein said plurality consists of at least 1000 of the genes corresponding to the markers listed in Table 1.

7. The method of claim 1, wherein said plurality consists of each of the genes corresponding to the 2,460 markers listed in Table 2.

8. The method of claim 1, wherein said plurality consists of the 550 gene markers listed in Table 2.

9. The method of claim 1, wherein said control comprises nucleic acids derived from a pool of tumors from individual sporadic patients.

10. The method of claim 1, wherein said detecting comprises the steps of

(a) generating an ER(+) template by hybridization of nucleic acids derived from a plurality of ER(+) patients within a plurality of sporadic patients against nucleic acids derived from a pool of tumors from individual sporadic patients;

(b) generating an ER(-) template by hybridization of nucleic acids derived from a plurality of ER(-) patients within said plurality of sporadic patients against nucleic acids derived from said pool of tumors from individual sporadic patients within said plurality;

(c) hybridizing an nucleic acids derived from an individual sample against said pool; and

(d) determining the similarity of marker gene expression in the individual sample to the ER(+) template and the ER(-) template, wherein if said expression is more similar to the ER(+) template, the sample is classified as ER(+), and if said expression is more similar to the ER(-) template, the sample is classified as ER(-).

11. A method for classifying a cell sample as *BRACA1*-related or sporadic, comprising detecting a difference in the expression of a first plurality of genes relative to a control, said first plurality of genes consisting of at least 5 of the genes corresponding to the markers listed in Table 3.

12. The method of claim 11, wherein said plurality consists of at least 50 of the genes corresponding to the markers listed in Table 3.

13. The method of claim 11, wherein said plurality consists of at least 100 of the genes corresponding to the markers listed in Table 3.

14. The method of claim 11, wherein said plurality consists of at least 200 of the genes corresponding to the markers listed in Table 3.

15. The method of claim 11, wherein said plurality consists of each of the genes corresponding to the 430 markers listed in Table 3.

16. The method of claim 11, wherein said plurality consists of each of the genes corresponding to the 100 markers listed in Table 4.

17. The method of claim 11, wherein said control comprises nucleic acids derived from a pool of tumors from individual sporadic patients.

18. The method of claim 11, wherein said detecting comprises the steps of

(a) generating a *BRCA1* template by hybridization of nucleic acids derived from a plurality of *BRCA1* patients within a plurality of ER(-) patients against nucleic acids derived from a pool of tumors;

(b) generating a sporadic template by hybridization of nucleic acids derived from a plurality of sporadic patients within said plurality of ER(-) patients against nucleic acids derived from said pool of tumors;

(c) hybridizing nucleic acids derived from an individual sample against said pool; and

(d) determining the similarity of marker gene expression in the individual sample to the*BRCA1* template and the sporadic template, wherein if said expression is more similar to the *BRCA1* template, the sample is classified as *BRCA1*, and if said expression is more similar to the sporadic template, the sample is classified as sporadic.

19. A method for classifying an individual as having a good prognosis (no distant metastases within five years of initial diagnosis) or a poor prognosis (distant metastases within five years of initial diagnosis), comprising detecting a difference in the expression of a first plurality of genes in a cell sample taken from the individual relative to a control, said first plurality of genes consisting of at least 5 of the genes corresponding to the markers listed in Table 5.

20. The method of claim 19, wherein said plurality consists of at least 20 of the genes corresponding to the markers listed in Table 5.

21. The method of claim 19, wherein said plurality consists of at least 100 of the genes corresponding to the markers listed in Table 5.

22. The method of claim 19, wherein said plurality consists of at least 150 of the genes corresponding to the markers listed in Table 5.

23. The method of claim 19, wherein said plurality consists of each of the genes corresponding to the 231 markers listed in Table 5.

24. The method of claim 19, wherein said plurality consists of the 70 gene markers listed in Table 6.

25. The method of claim 1, wherein said control comprises nucleic acids derived from a pool of tumors from individual sporadic patients.

26. The method of claim 19, wherein said detecting comprises the steps of:

(a) generating a good prognosis template by hybridization of nucleic acids derived from a plurality of good prognosis patients against nucleic acids derived from a pool of tumors from individual patients;

(b) generating a poor prognosis template by hybridization of nucleic acids derived from a plurality of poor prognosis patients against nucleic acids derived from said pool of tumors from said plurality of individual patients;

(c) hybridizing an nucleic acids derived from and individual sample against said pool; and

(d) determining the similarity of marker gene expression in the individual sample to the good prognosis template and the poor prognosis template, wherein if said expression is more similar to the good prognosis template, the sample is classified as having a good prognosis, and if said expression is more similar to the poor prognosis template, the sample is classified as having a poor prognosis.

27. The method of claim 1, wherein the cell sample is additionally classified as *BRCA1*-related or sporadic by detecting a difference in the expression of a second plurality of genes in a cell sample taken from the individual relative to a control, said second plurality of genes consisting of at least 5 of the genes corresponding to the markers listed in Table 3 or Table 4.

28. The method of claim 1, wherein the cell sample is additionally classified as taken from a patient with a good prognosis or a poor prognosis by detecting a difference in the expression of a second plurality of genes in a cell sample taken from the individual relative to a control, said second plurality of genes consisting of at least 5 of the genes corresponding to the markers listed in Table 5.

29. The method of claim 11, wherein the cell sample is additionally classified as taken from a patient with a good prognosis or a poor prognosis by detecting a difference in the expression of a second plurality of genes in a cell sample taken from the individual relative to a control, said second plurality of genes consisting of at least 20 of the genes corresponding to the markers listed in Table 5.

30. The method of claim 11, wherein the cell sample is additionally classified as ER(+) or ER(-) by detecting a difference in the expression of a second plurality of genes in a cell sample taken from the individual relative to a control, said second plurality of genes consisting of at least 5 of the genes corresponding to the markers listed in Table 1.

31. The method of claim 19, wherein the cell sample is additionally classified as ER(+) or ER(-) by detecting a difference in the expression of a second plurality of genes in a cell sample taken from the individual relative to a control, said second plurality of genes consisting of at least 5 of the genes corresponding to the markers listed in Table 1.

32. The method of claim 19, wherein the cell sample is additionally classified as *BRCA1* or sporadic by detecting a difference in the expression of a second plurality of genes in a cell sample taken from the individual relative to a control, said second plurality of genes consisting of at least 5 of the genes corresponding to the markers listed in Table 3.

33. A method for classifying a sample as ER(+) or ER(-) by calculating the similarity between the expression of at least 5 of the markers listed in Table 1 in the sample to the expression of the same markers in an ER(-) nucleic acid pool and an ER(+) nucleic acid pool, comprising the steps of:

(a) labeling nucleic acids derived from a sample, with a first fluorophore to obtain a first pool of fluorophore-labeled nucleic acids;

(b) labeling with a second fluorophore a first pool of nucleic acids derived from two or more ER(+) samples, and a second pool of nucleic acids derived from two or more ER(-) samples:

(c) contacting said first fluorophore-labeled nucleic acid and said first pool of second fluorophore-labeled nucleic acid with a first microarray under conditions

such that hybridization can occur, and contacting said first fluorophore-labeled nucleic acid and said second pool of second fluorophore-labeled nucleic acid with a second microarray under conditions such that hybridization can occur, wherein said first microarray and said second microarray are similar to each other, exact replicas of each other, or are identical, detecting at each of a plurality of discrete loci on the first microarray a first flourescent emission signal from said first fluorophore-labeled nucleic acid and a second fluorescent emission signal from said first pool of second fluorophore-labeled genetic matter that is bound to said first microarray under said conditions, and detecting at each of the marker loci on said second microarray said first fluorescent emission signal from said first fluorophore-labeled nucleic acid and a third fluorescent emission signal from said second pool of second fluorophore-labeled nucleic acid;

(d) determining the similarity of the sample to the ER(-) and ER(+) pools by comparing said first fluorescence emission signals and said second fluorescence emission signals, and said first emission signals and said third fluorescence emission signals; and

(e) classifying the sample as ER(+) where the first fluorescence emission signals are more similar to said second fluorescence emission signals than to said third fluorescent emission signals, and classifying the sample as ER(-) where the first fluorescence emission signals are more similar to said third fluorescence emission signals than to said second fluorescent emission signals.

34. The method of claim 33, wherein said similarity is calculated by determining a first sum of the differences of expression levels for each marker between said first fluorophore-labeled nucleic acid and said first pool of second fluorophore-labeled nucleic acid, and a second sum of the differences of expression levels for each marker between said first fluorophore-labeled nucleic acid and said second pool of second fluorophore-labeled nucleic acid, wherein if said first sum is greater than said second sum, the sample is classified as ER(-), and if said second sum is greater than said first sum, the sample is classified as ER(+).

35. The method of claim 33, wherein said similarity is calculated by computing a first classifier parameter P_1 between an ER(+) template and the expression of said markers in said sample, and a second classifier parameter P_2 between an ER(-) template and the expression of said markers in said sample, wherein said P_1 and P_2 are calculated according to the formula:

$$P_i = (\vec{z}_i \bullet \vec{y}) / (\|\vec{z}_i\| \cdot \|\vec{y}\|),$$

wherein $\vec{z}_1$ and $\vec{z}_2$ are ER(+) and ER(-) templates, respectively, and are calculated by averaging said second fluorescence emission signal for each of said markers in said first pool of second fluorophore-labeled nucleic acid and said third fluorescence emission signal for each of said markers in said second pool of second fluorophore-labeled nucleic acid, respectively, and wherein $\vec{y}$ is said first fluorescence emission signal of each of said markers in the sample to be classified as ER(+) or ER(-), wherein the expression of the markers in the sample is similar to ER(-) if $P_1 < P_2$, and similar to ER(+) if $P_1 > P_2$.

36. A method for determining a set of marker genes whose expression is associated with a particular phenotype, comprising the steps of:

(a) selecting phenotype having two or more phenotype categories;

(b) identifying a plurality of genes wherein the expression of said genes is correlated or anticorrelated with one of the phenotype categories, and wherein the correlation coefficient for each gene is calculated according to the equation $\rho = (\vec{c} \bullet \vec{r}) / (\|\vec{c}\| \cdot \|\vec{r}\|)$, wherein $\vec{c}$ is a number representing said phenotype category and $\vec{r}$ is the logarithmic expression ratio across all the samples for each individual gene, wherein if the correlation coefficient has an absolute value of 0.3 or greater, said expression of said gene is associated with the phenotype category,

wherein said plurality of genes is a set of marker genes whose expression is associated with a particular phenotype.

37. The method of claim 36, wherein said set of marker genes is validated by:

(a) using a statistical method to randomize the association between said marker genes and said phenotype category, thereby creating a control correlation coefficient for each marker gene;

(b) repeating step (a) one hundred or more times to develop a frequency distribution of said control correlation coefficients for each marker gene;

(c) determining the number of marker genes having a control correlation coefficient of 0.3 or above, thereby creating a control marker gene set; and

(d) comparing the number of control marker genes so identified to the number of marker genes, wherein if the p value of the difference between the number of marker genes and the number of control genes is less than a threshold, said set of marker genes is validated.

38. The method of claim 36, wherein said set of marker genes is optimized by the method comprising:

(a) rank-ordering the genes by amplitude of correlation or by significance of the correlation coefficients to create a rank-ordered list, and

(b) selecting an arbitrary number n of marker genes from the top of the rank-ordered list.

39. The method of claim 38, wherein said set of marker genes is further optimized by the method comprising:

(a) calculating an error rate for said arbitrary number n of marker genes;

(b) increasing by 1 the number of genes selected from the top of the rank-ordered list;

(c) calculating an error rate for said number of genes selected from the top of the rank-ordered list;

(d) repeating steps (b) and (c) until said number of genes selected from the top of the rank-ordered list includes all genes included in said rank ordered list, and

(e) identifying said number of genes selected from the top of the rank-ordered list for which the error rate is smallest,

wherein said set of marker genes is optimized when the error rate is the smallest.

40. A method for assigning a person to one of a plurality of categories in a clinical trial, comprising determining for each said person the level of expression of at least five of the prognosis markers listed in Table 6, determining therefrom whether the person has an expression pattern that correlates with a good prognosis or a poor prognosis, and assigning said person to one category in a clinical trial if said person is determined to have a good prognosis, and a different category if that person is determined to have a poor prognosis.

41. A method of classifying a first cell or organism as having one of at least two different phenotypes, said at least two different phenotypes comprising a first phenotype and a second phenotype, said method comprising:

(a) comparing the level of expression of each of a plurality of genes in a first sample from the first cell or organism to the level of expression of each of said genes, respectively, in a pooled sample from a plurality of cells or organisms, said plurality of cells or organisms comprising different cells or organisms exhibiting said at least two different phenotypes, respectively, to produce a first compared value;

(b) comparing said first compared value to a second compared value, wherein said second compared value is the product of a method comprising comparing the level of expression of each of said genes in a sample from a cell or organism characterized as having said first phenotype to the level of expression of each of said genes, respectively, in said pooled sample;

(c) comparing said first compared value to a third compared value, wherein said third compared value is the product of a method comprising comparing the level of expression of each of said genes in a sample from a cell or organism characterized as having said second phenotype to the level of expression of each of said genes, respectively, in said pooled sample,

(d) optionally carrying out one or more times a step of comparing said first compared value to one or more additional compared values, respectively, each additional compared value being the product of a method comprising comparing the level of expression of each of said genes in a sample from a cell or organism characterized as having a phenotype different from said first and second phenotypes but included among said at least two different phenotypes, to the level of expression of each of said genes, respectively, in said pooled sample; and

(e) determining to which of said second, third and, if present, one or more additional compared values, said first compared value is most similar;

wherein said first cell or organism is determined to have the phenotype of the cell or organism used to produce said compared value most similar to said first compared value.

42. The method of claim 40, wherein said compared values are each ratios of the levels of expression of each of said genes.

43. The method of claim 40, wherein each of said levels of expression of each of said genes in said pooled sample are normalized prior to any of said comparing steps.

44. The method of claim 42 wherein normalizing said levels of expression is carried out by dividing each of said levels of expression by the median or mean level of expression of each of said genes or dividing by the mean or median level of expression of one or more housekeeping genes in said pooled sample.

45. The method of claim 42 wherein said normalized levels of expression are subjected to a log transform and said comparing steps comprise subtracting said log transform from the log of said levels of expression of each of said genes in said sample from said cell or organism.

46. The method of claim 40, wherein said at least two different phenotypes are different stages of a disease or disorder.

47. The method of claim 40, wherein said at least two different phenotypes are different prognoses of a disease or disorder.

48. The method of claim 40, wherein said levels of expression of each of said genes, respectively, in said pooled sample or said levels of expression of each of said genes in a sample from said cell or organism characterized as having said first phenotype, said second phenotype, or said phenotype different from said first and second phenotypes, respectively, are stored on a computer.

49. A microarray comprising at least 5 markers derived from any one of Tables 1-6, wherein at least 50% of the probes on the microarray are present in any one of Tables 1-6.

50. The microarray of claim 48, wherein at least 70% of the probes on the microarray are present in any one of Tables 1-6.

51. The microarray of claim 48, wherein at least 80% of the probes on the microarray are present in any one of Tables 1-6.

52. The microarray of claim 48, wherein at least 90% of the probes on the microarray are present in any one of Tables 1-6.

53. The microarray of claim 48, wherein at least 95% of the probes on the microarray are present in any one of Tables 1-6.

54. The microarray of claim 48, wherein at least 98% of the probes on the microarray are present in any one of Tables 1-6.

55. A microarray for distinguishing ER(+) and ER(-) cell samples comprising a positionally-addressable array of polynucleotide probes bound to a support, said polynucleotide probes comprising a plurality of polynucleotide probes of different nucleotide sequences, each of said different nucleotide sequences comprising a sequence complementary and hybridizable to a different gene, said plurality consisting of at least 20 of the genes corresponding to the markers listed in Table 1 or Table 2, wherein at least 50% of the probes on the microarray are present in Table 1 or Table 2.

56. A microarray for distinguishing *BRCA1*-related and sporadic cell samples comprising a positionally-addressable array of polynucleotide probes bound to a support, said polynucleotide probes comprising a plurality of polynucleotide probes of different nucleotide sequences, each of said different nucleotide sequences comprising a sequence complementary and hybridizable to a different gene, said plurality consisting of at least 20 of the genes corresponding to the markers listed in Table 3 or Table 4, wherein at least 50% of the probes on the microarray are present in Table 3 or Table 4.

57. A microarray for distinguishing cell samples from individuals having a good prognosis and cell samples from individuals having a poor prognosis, comprising a positionally-addressable array of polynucleotide probes bound to a support, said polynucleotide probes comprising a plurality of polynucleotide probes of different nucleotide sequences, each of said different nucleotide sequences comprising a sequence complementary and hybridizable to a different, said plurality consisting of at least 20 of the genes corresponding to the markers listed in Table 5 or Table 6, wherein at least 50% of the probes on the microarray are present in Table 5 or Table 6.

58. A kit for determining whether a sample contains a *BRCA1* or sporadic mutation, comprising at least one microarray comprising probes to at least 20 of the genes corresponding to the markers listed in Table 3, and a computer readable medium having recorded thereon one or more programs for determining the similarity of the level of nucleic acid derived from the markers listed in Table 3 in a sample to that in a *BRCA1* pool and a sporadic tumor pool, wherein the one or more programs cause a computer to perform a method comprising computing the aggregate differences in expression of each marker between the sample and *BRCA1* and the aggregate differences in expression of each marker between the sample and sporadic pool, or a method comprising determining the correlation of expression of the markers in the sample to the expression in the *BRCA1* and sporadic pools, said correlation calculated according to Equation (3).

59. A kit for determining the ER-status of a sample, comprising at least one microarray comprising probes to at least 20 of the genes corresponding to the markers listed in Table 1, and a computer readable medium having recorded thereon one or more programs for determining the similarity of the level of nucleic acid derived from the markers listed in Table 1 in a sample to that in an ER(-) pool and an ER(+) pool, wherein the one or more programs cause a computer to perform a method comprising computing the aggregate differences in expression of each marker between the sample and ER(-) pool and the aggregate differences in expression of each marker between the sample and ER(+) pool, or a method comprising determining the correlation of expression of the markers in the sample to the expression in the ER(-) and ER(+) pools, said correlation calculated according to Equation (3).

60. A kit for determining whether a sample is derived from a patient having a good prognosis or a poor prognosis, comprising at least one microarray comprising probes to at least 20 of the genes corresponding to the markers listed in Table 5, and a computer readable medium having recorded thereon one or more programs for determining the similarity of the level of nucleic acid derived from the markers listed in Table 5 in a sample to that in a pool of samples derived from individuals having a good prognosis and a pool of samples derived from individuals having a good prognosis, wherein the one or more programs cause a computer to perform a method comprising computing the aggregate differences in expression of each marker between the sample and the good prognosis pool and the aggregate differences in expression of each marker between the sample and the poor prognosis pool, or a method comprising determining the correlation of expression of the

markers in the sample to the expression in the good prognosis and poor prognosis pools, said correlation calculated according to Equation (3).

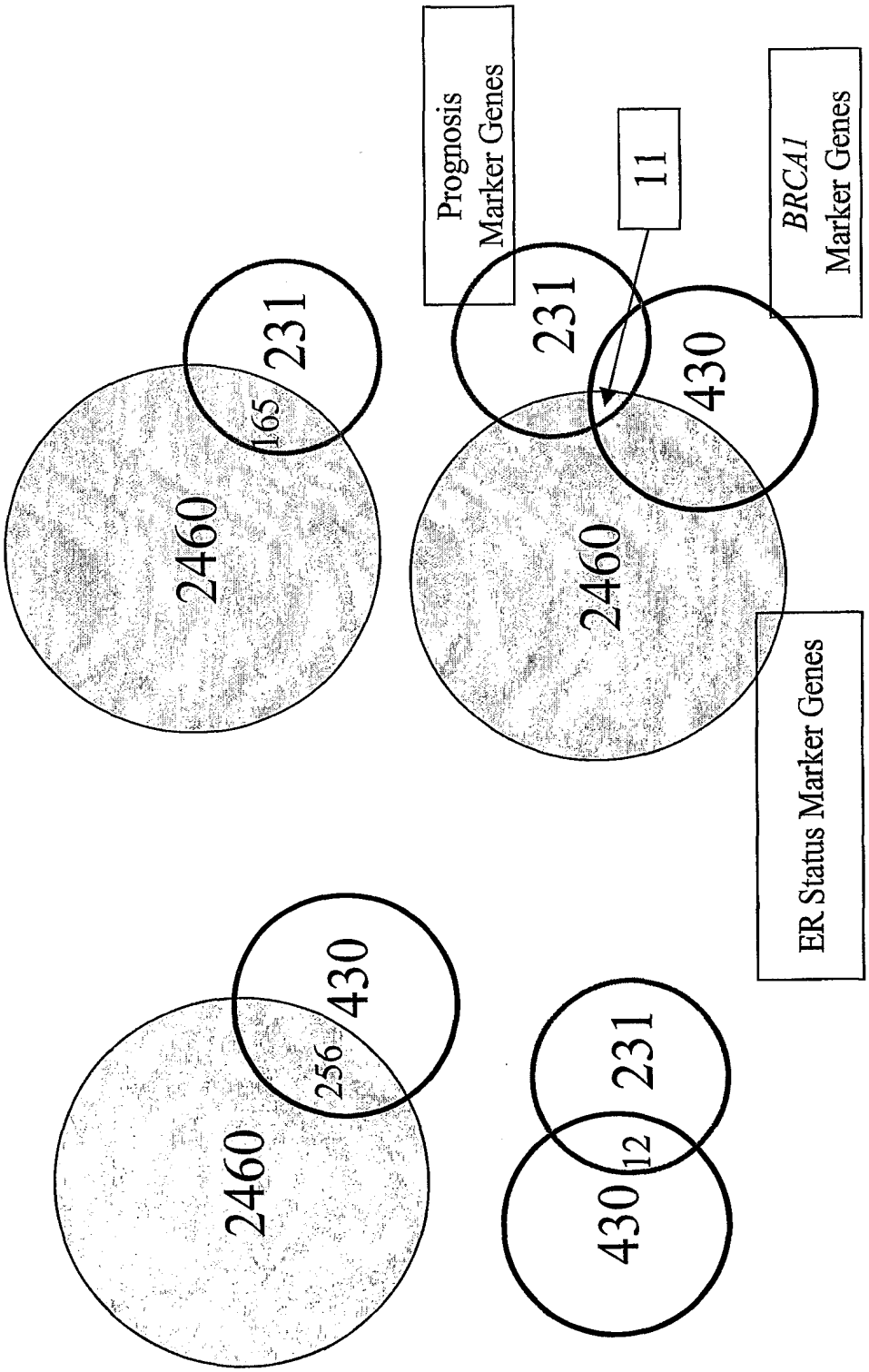
2460
165
231
Prognosis Marker Genes
231
11
2460
430
BRCA1 Marker Genes
ER Status Marker Genes
2460
256
430
430
12
231

FIG. 1

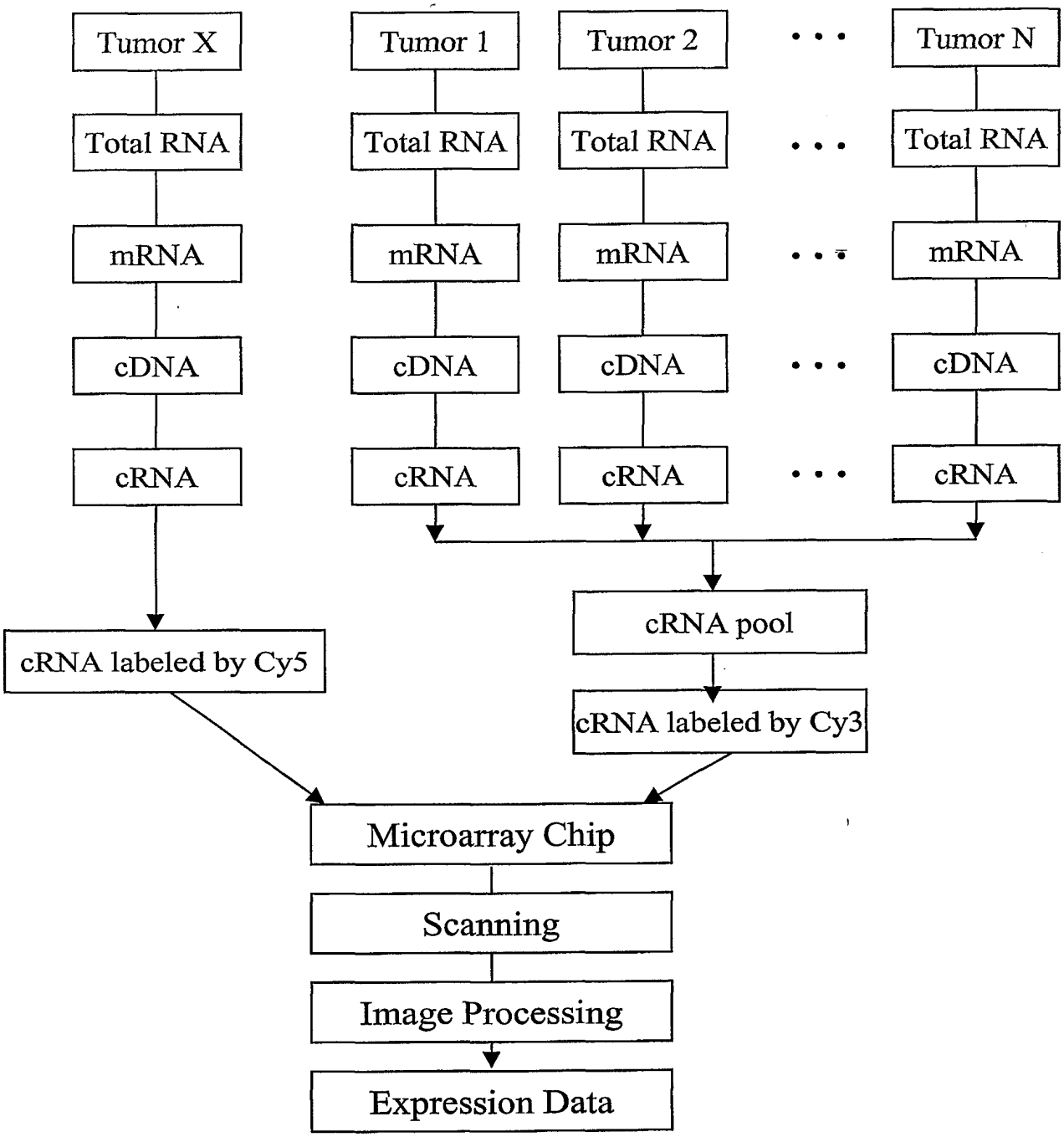
Tumor X
Tumor 1
Tumor 2
Tumor N
Total RNA
Total RNA
Total RNA
Total RNA
mRNA
mRNA
mRNA
mRNA
cDNA
cDNA
cDNA
cDNA
cRNA
cRNA
cRNA
cRNA
cRNA pool
cRNA labeled by Cy5
cRNA labeled by Cy3
Microarray Chip
Scanning
Image Processing
Expression Data

FIG. 2

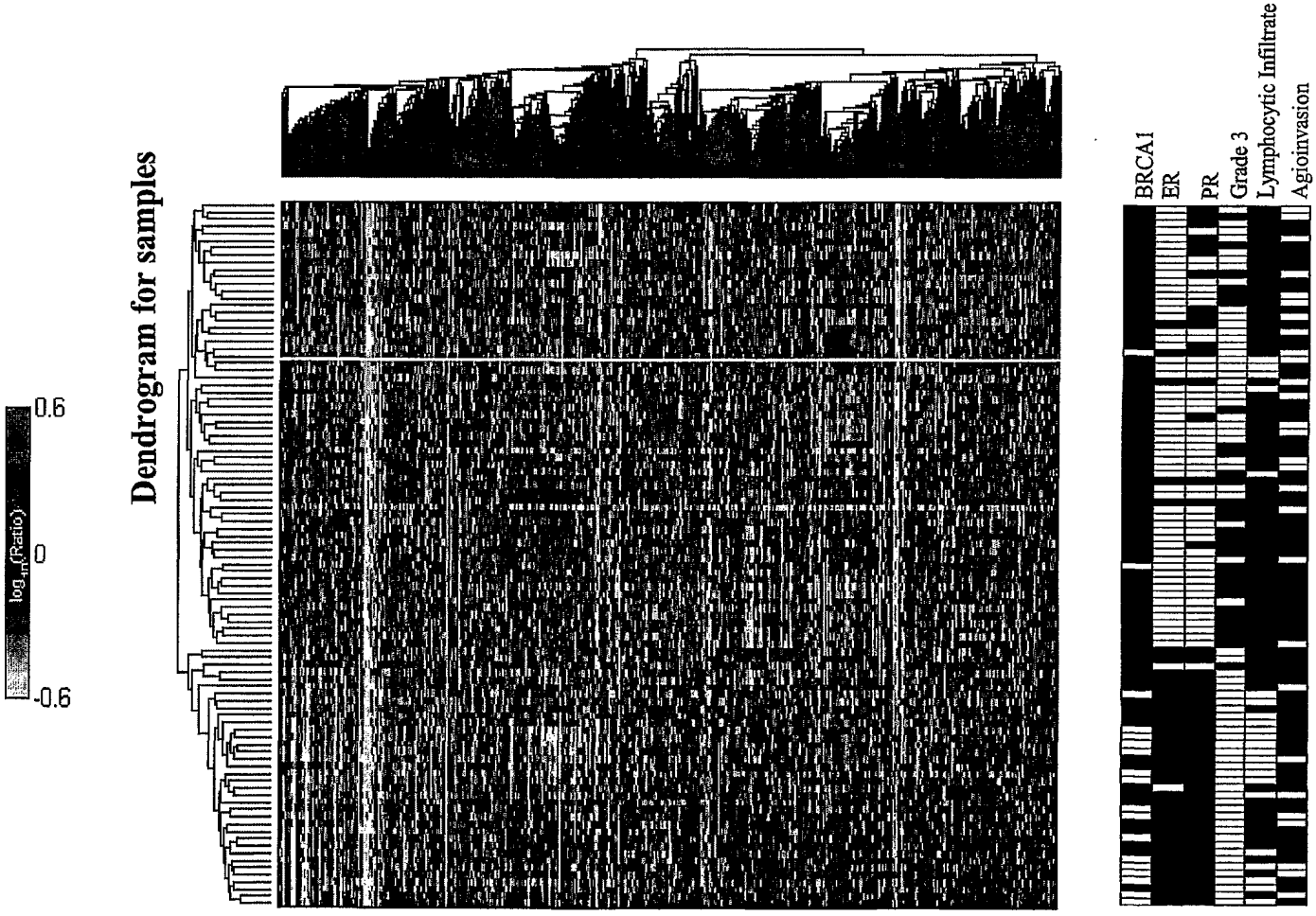
Dendrogram for samples
0.6
0
-0.6
log10(Ratio)
BRCA1
ER
PR
Grade 3
Lymphocytic Infiltrate
Agioinvasion

FIG. 3

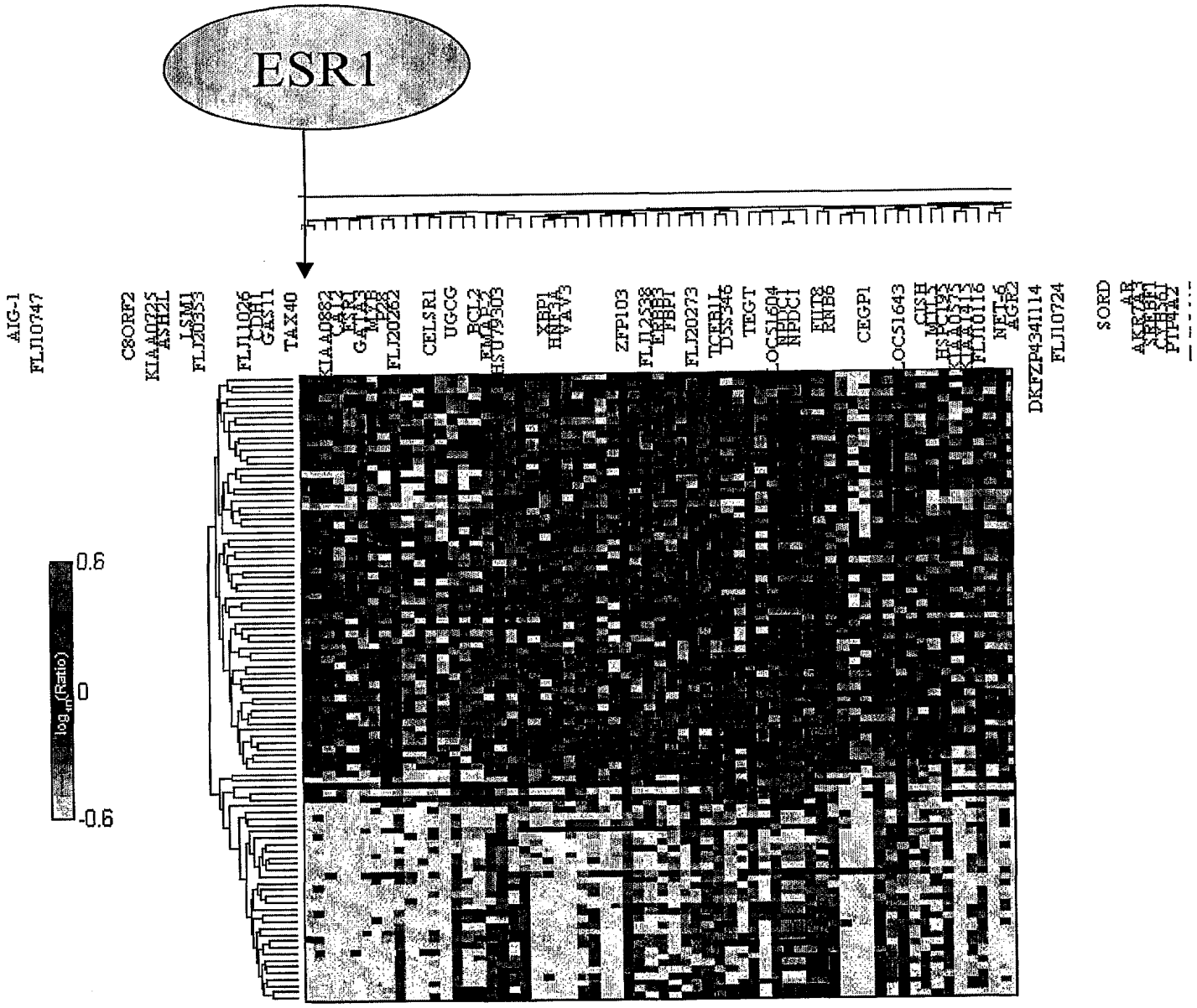
ESR1
AIG-1
FLJ10747
C8ORF2
KIAA0225
ASH2L
LSM1
FLJ20353
FLJ11026
GAS11
TAX40
KIAA0882
CA11
GATA3
MYB
FLJ20262
CELSR1
UGCG
BCL2
FWP009
HSU79303
XBP1
HOXA5
VAV3
ZFP103
FLJ12338
EMR3
FBP1
FLJ20273
TCEB1L
DSS346
TEGT
OCS1604
NPDC1
ENTB6
CEGP1
OCS1643
CISH
MCT93
HSPC052
KIAA0312
FLJ10116
NET-6
AGR2
DKFZP434I114
FLJ10724
SORD
AR
AKR7A3
SERBP1
CYB5R1
PTP4A2
0.6
0
-0.6
log2(Ratio)

FIG. 4

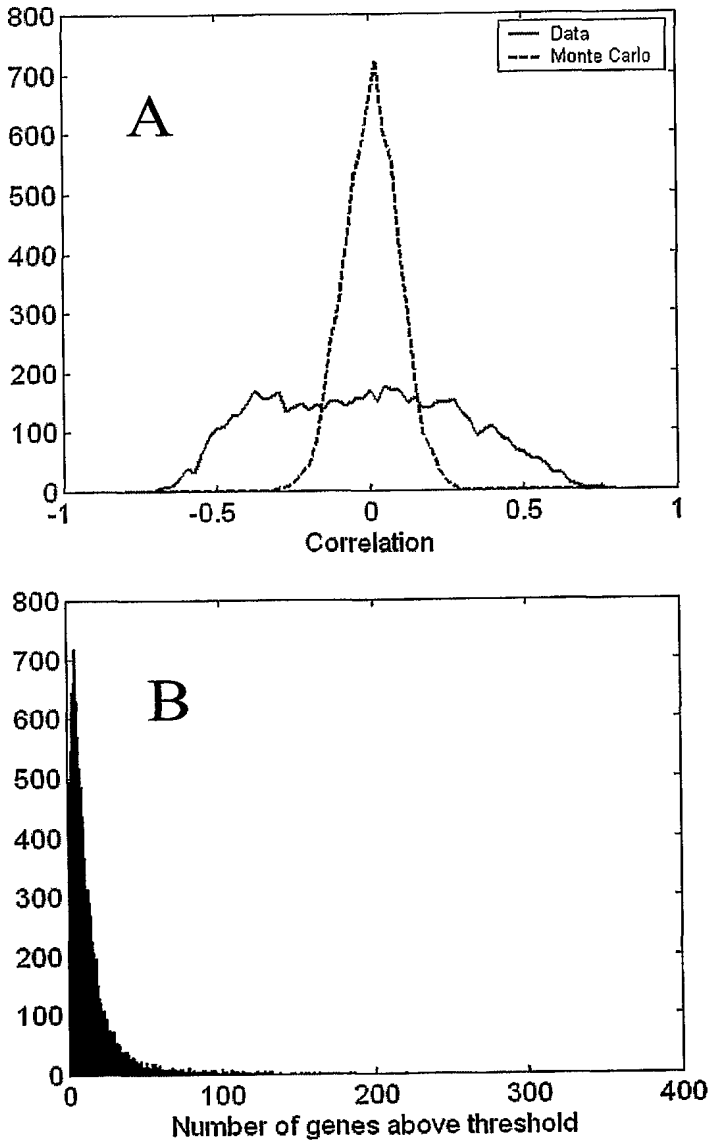
A
Data
Monte Carlo
800
700
600
500
400
300
200
100
0
-1
-0.5
0
0.5
1
Correlation
B
800
700
600
500
400
300
200
100
0
0
100
200
300
400
Number of genes above threshold

FIG. 5

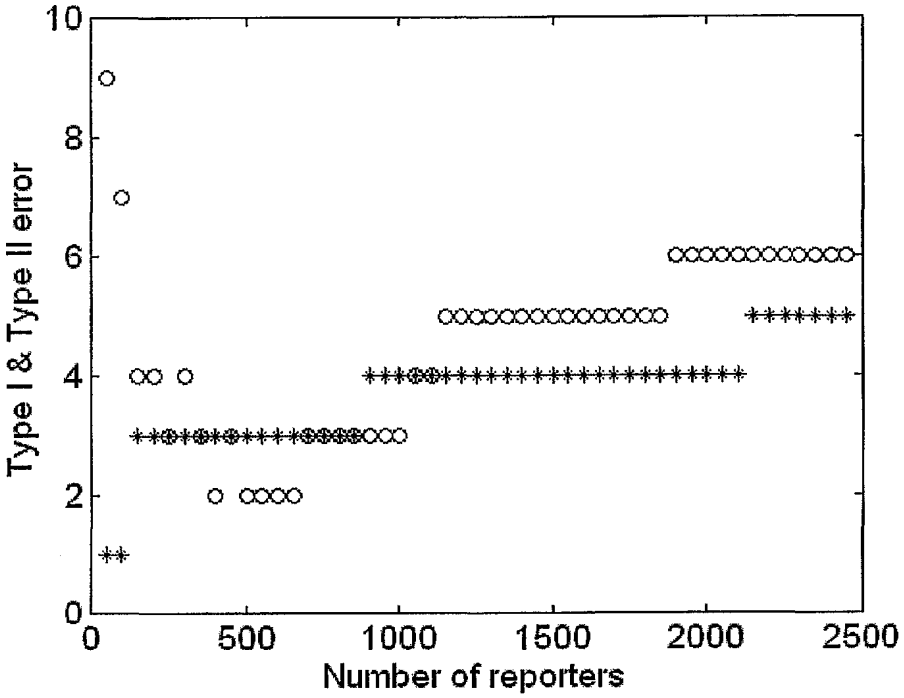
Type I & Type II error
Number of reporters
0
2
4
6
8
10
0
500
1000
1500
2000
2500

FIG. 6

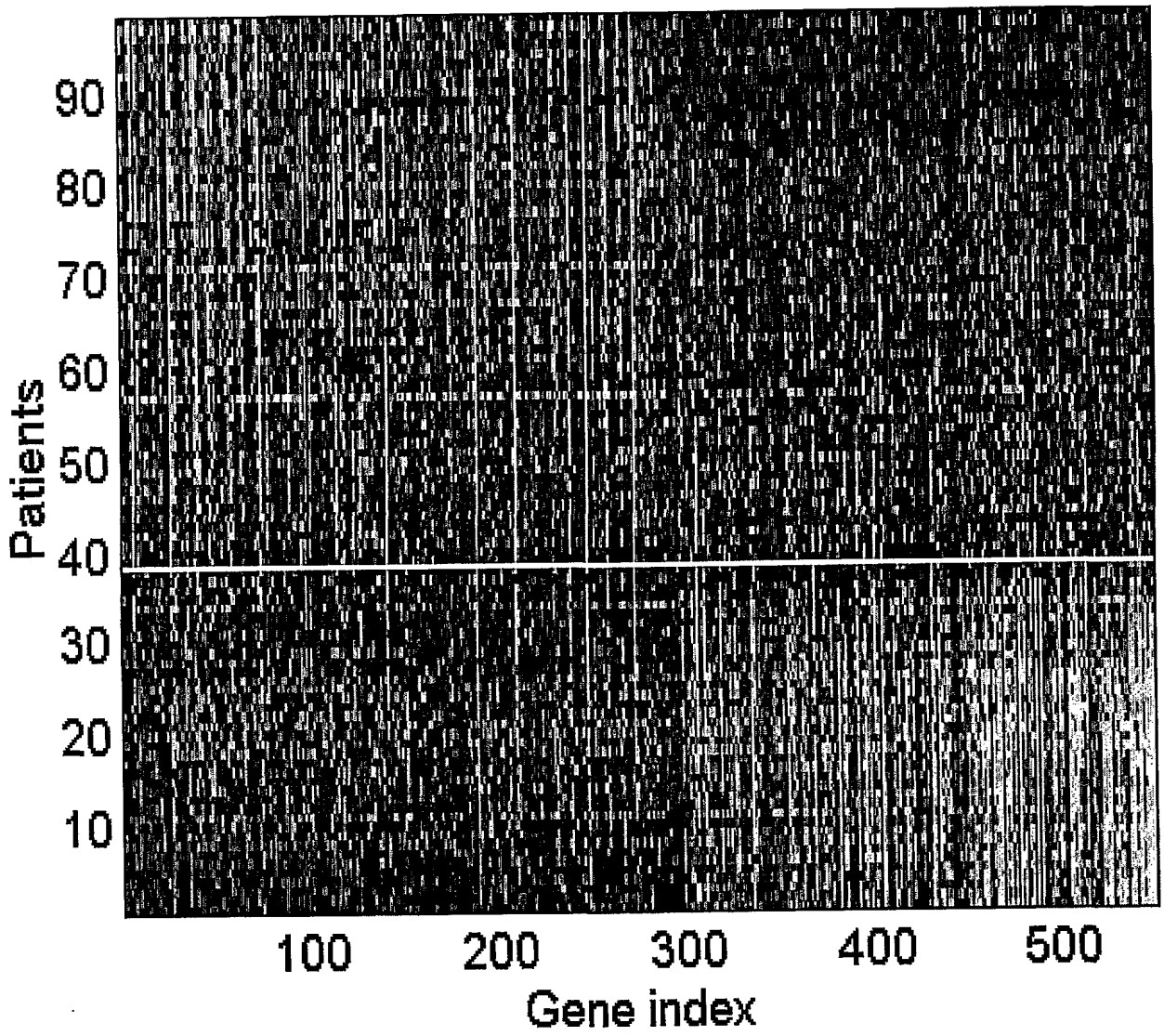
90
80
70
60
50
40
30
20
10
Patients
100
200
300
400
500
Gene index

FIG. 7

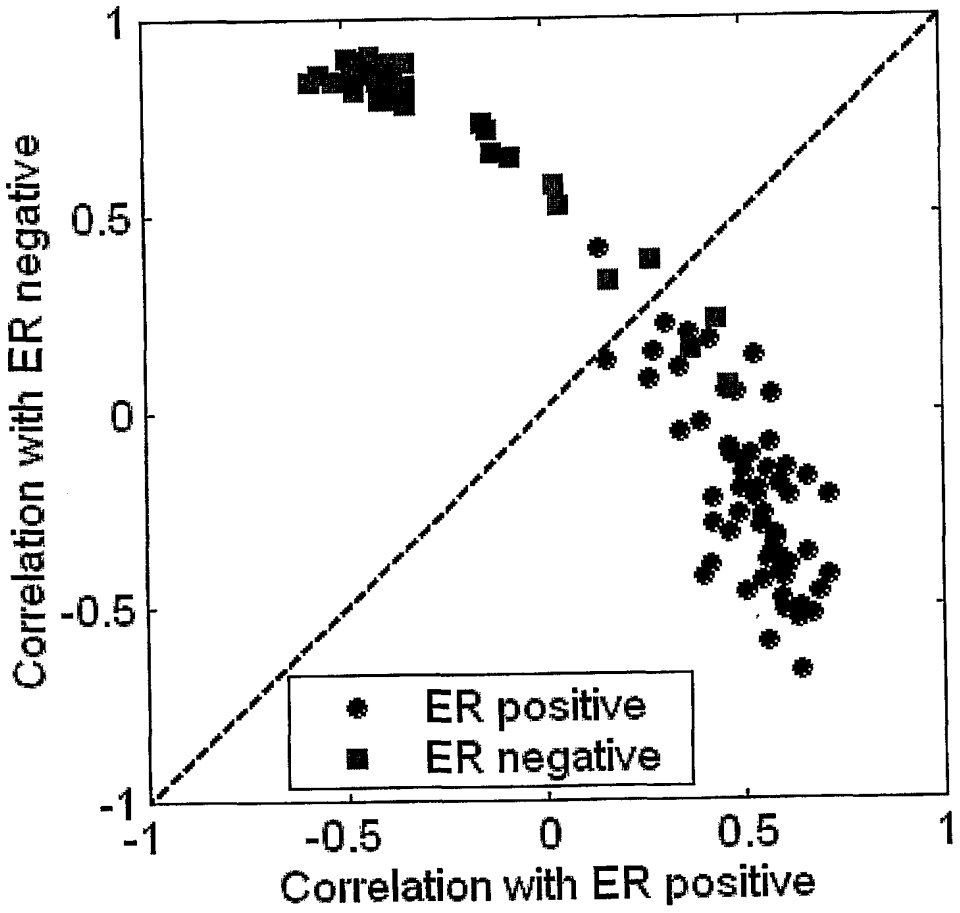
1
0.5
0
-0.5
-1
-1
-0.5
0
0.5
1
Correlation with ER negative
Correlation with ER positive
ER positive
ER negative

FIG. 8

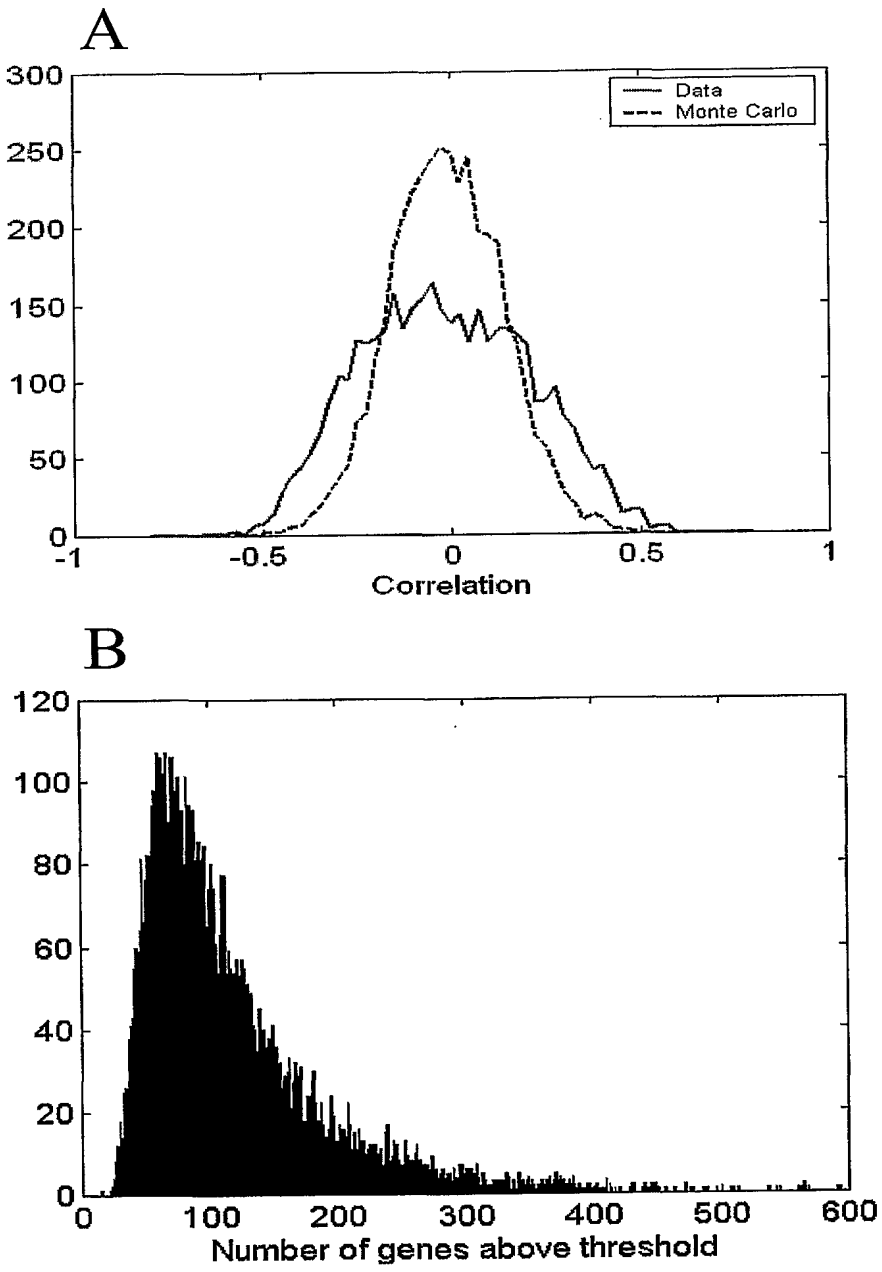
A
300
250
200
150
100
50
0
-1
-0.5
0
0.5
1
Correlation
Data
Monte Carlo
B
120
100
80
60
40
20
0
0
100
200
300
400
500
600
Number of genes above threshold

FIG. 9

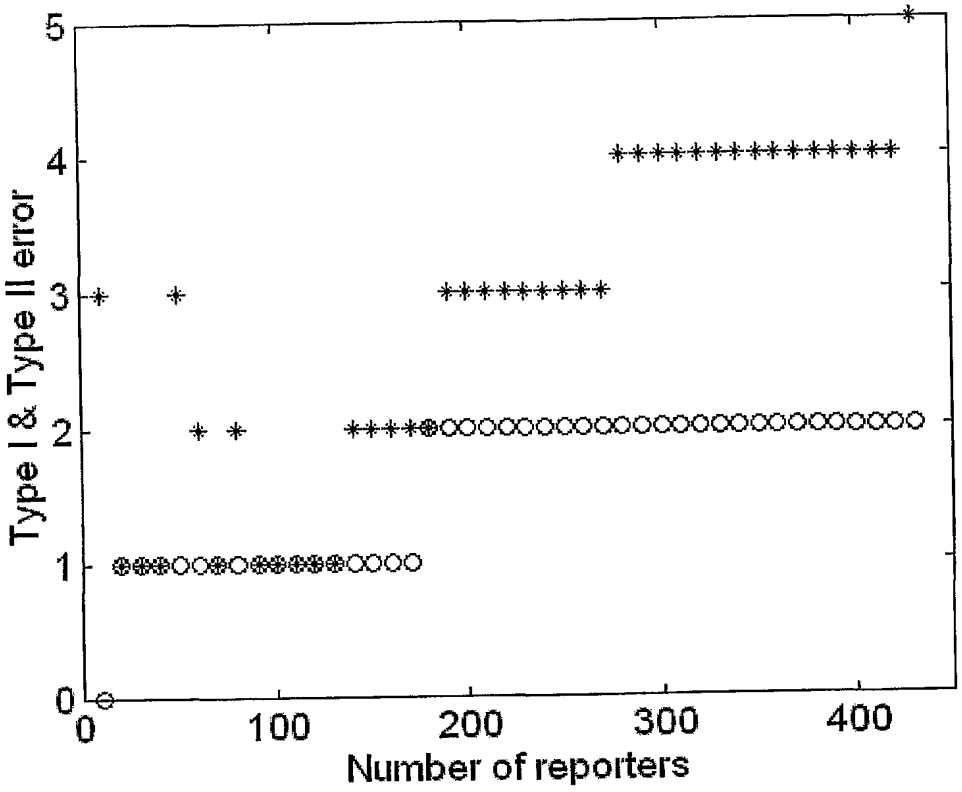
Type I & Type II error
Number of reporters
0
1
2
3
4
5
0
100
200
300
400

FIG. 10

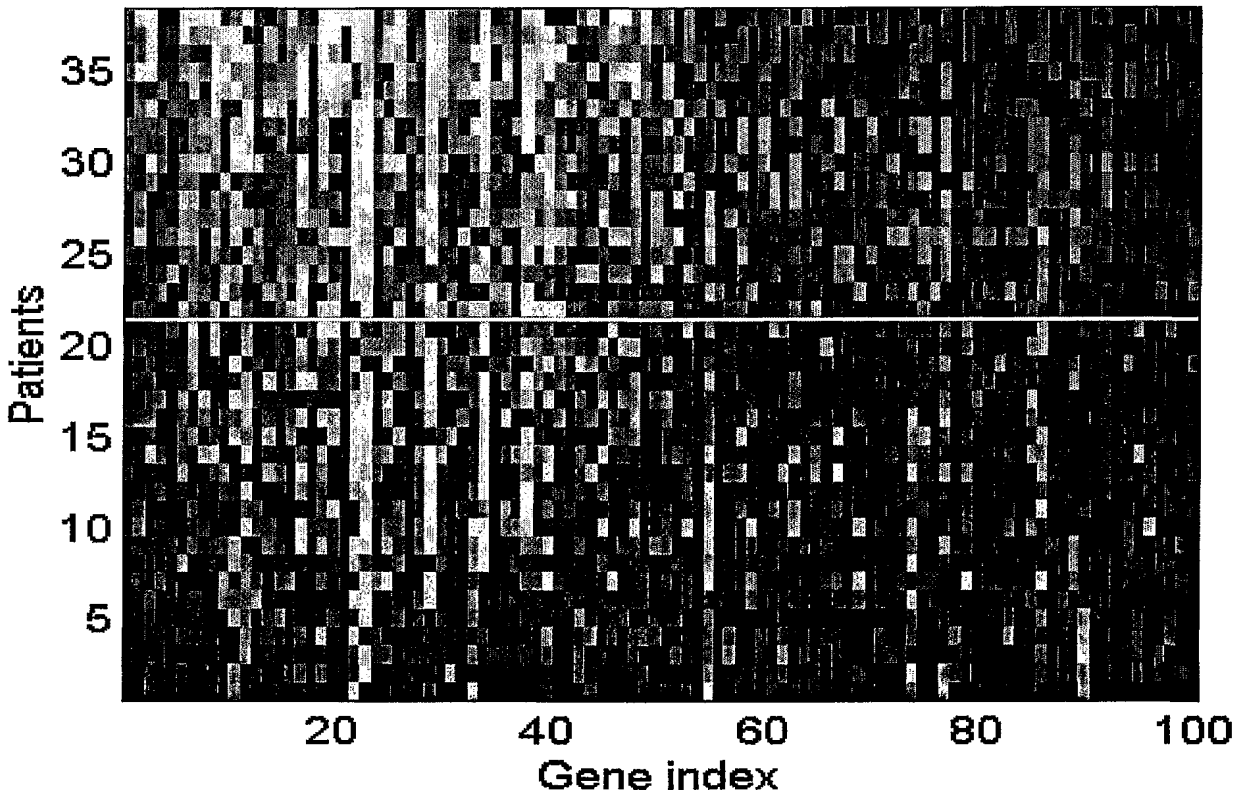

FIG. 11A

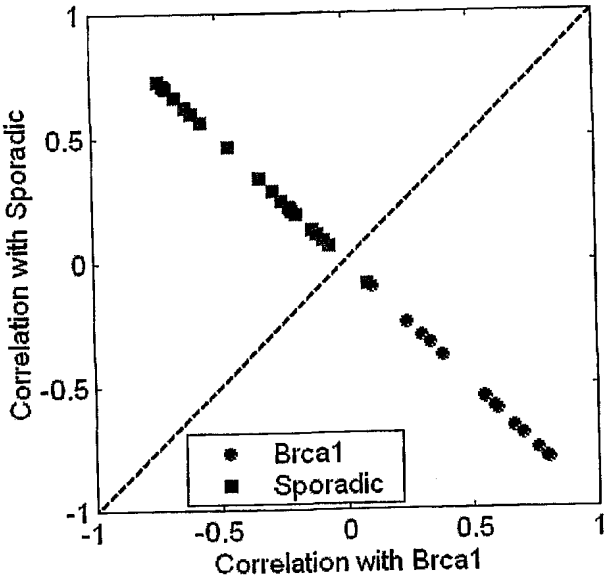
1
0.5
0
-0.5
-1
-1
-0.5
0
0.5
1
Correlation with Sporadic
Correlation with Brca1
Brca1
Sporadic

FIG. 11B

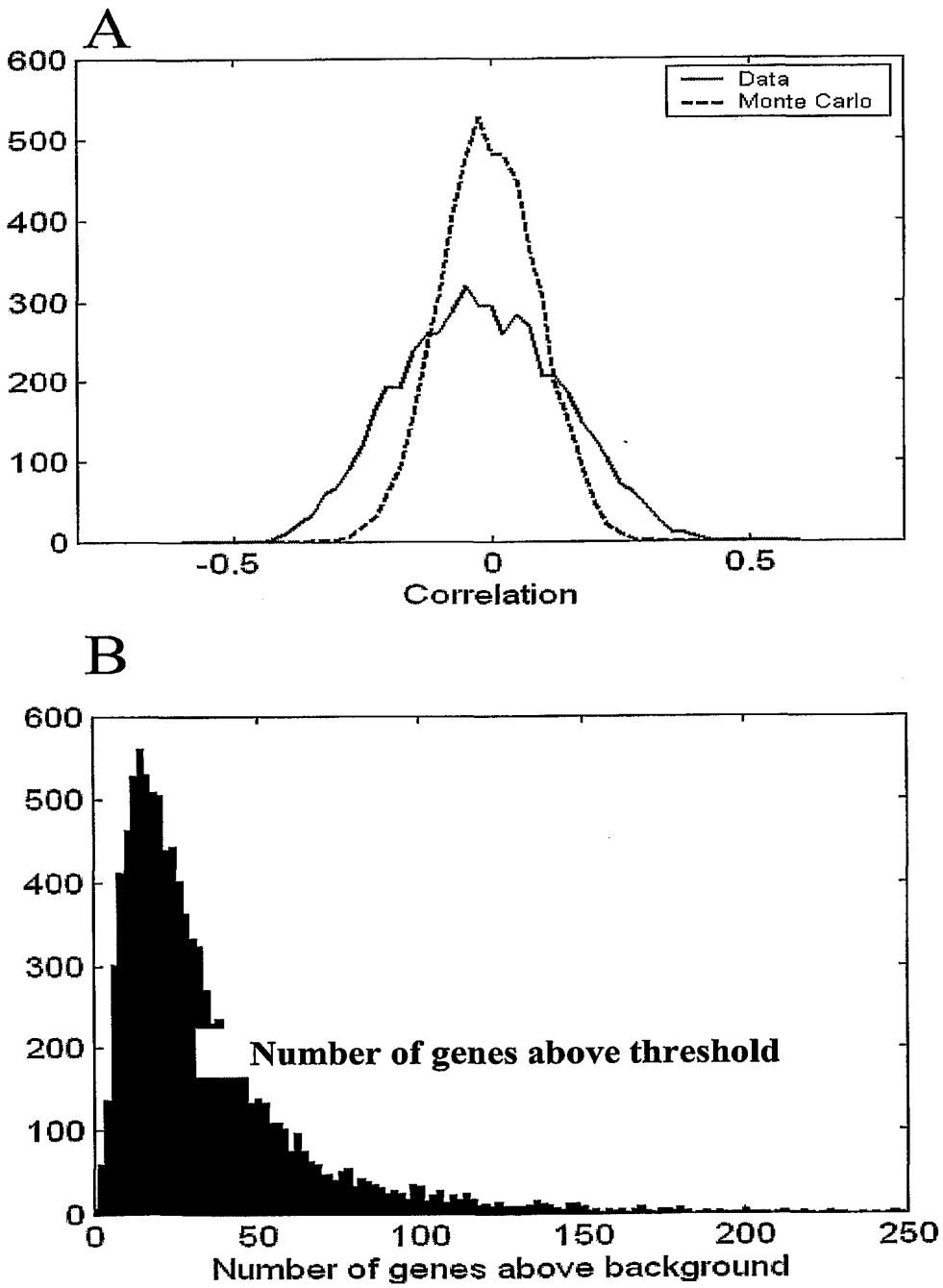
A
600
500
400
300
200
100
0
-0.5
0
0.5
Correlation
Data
Monte Carlo
B
600
500
400
300
200
100
0
0
50
100
150
200
250
Number of genes above threshold
Number of genes above background

FIG. 12

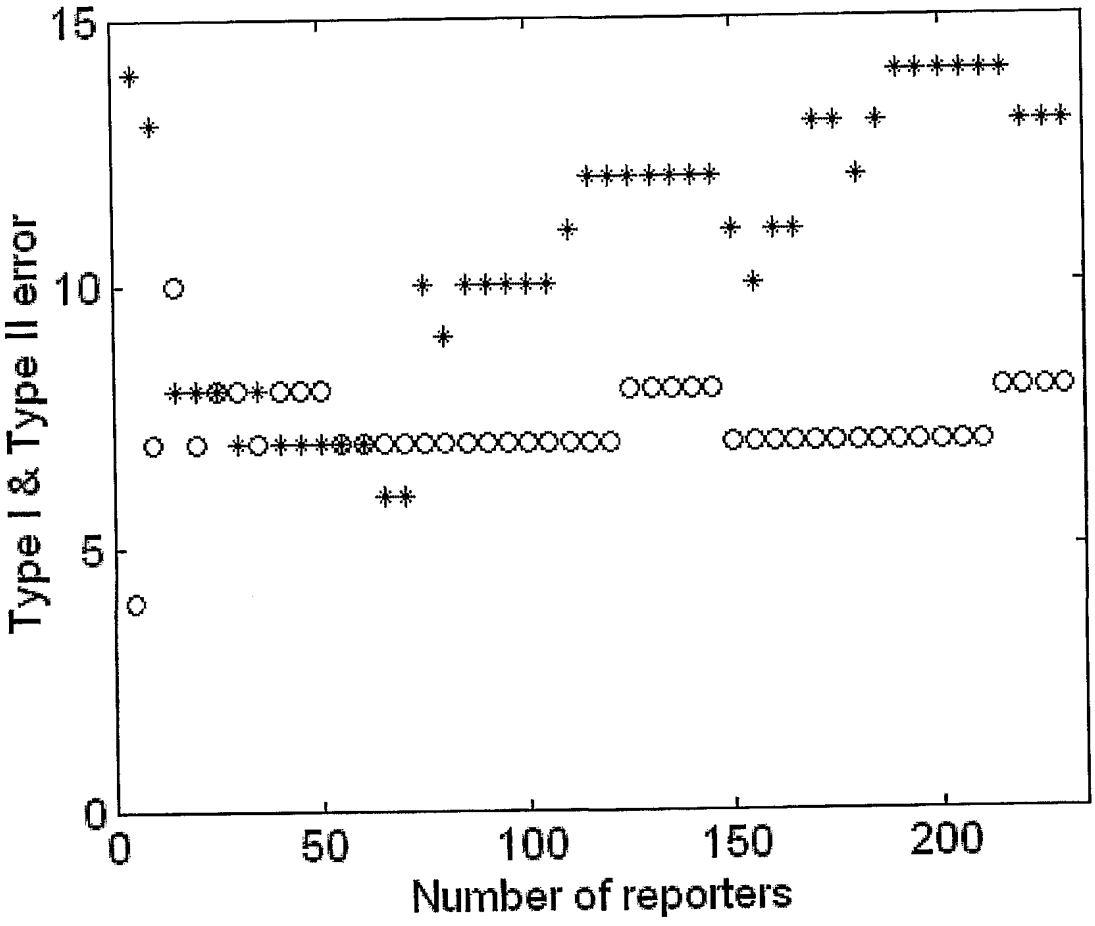
15
10
5
0
Type I & Type II error
0
50
100
150
200
Number of reporters

FIG. 13

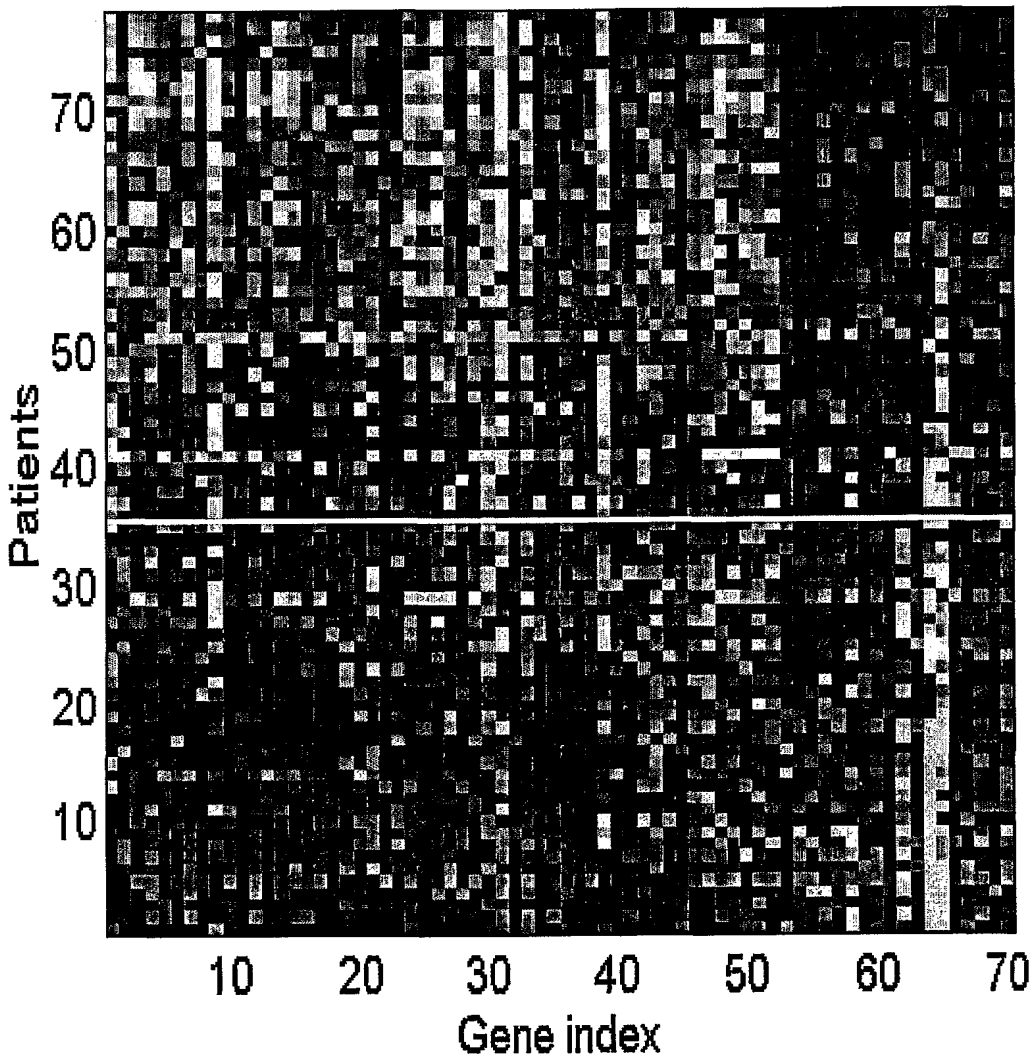

FIG. 14

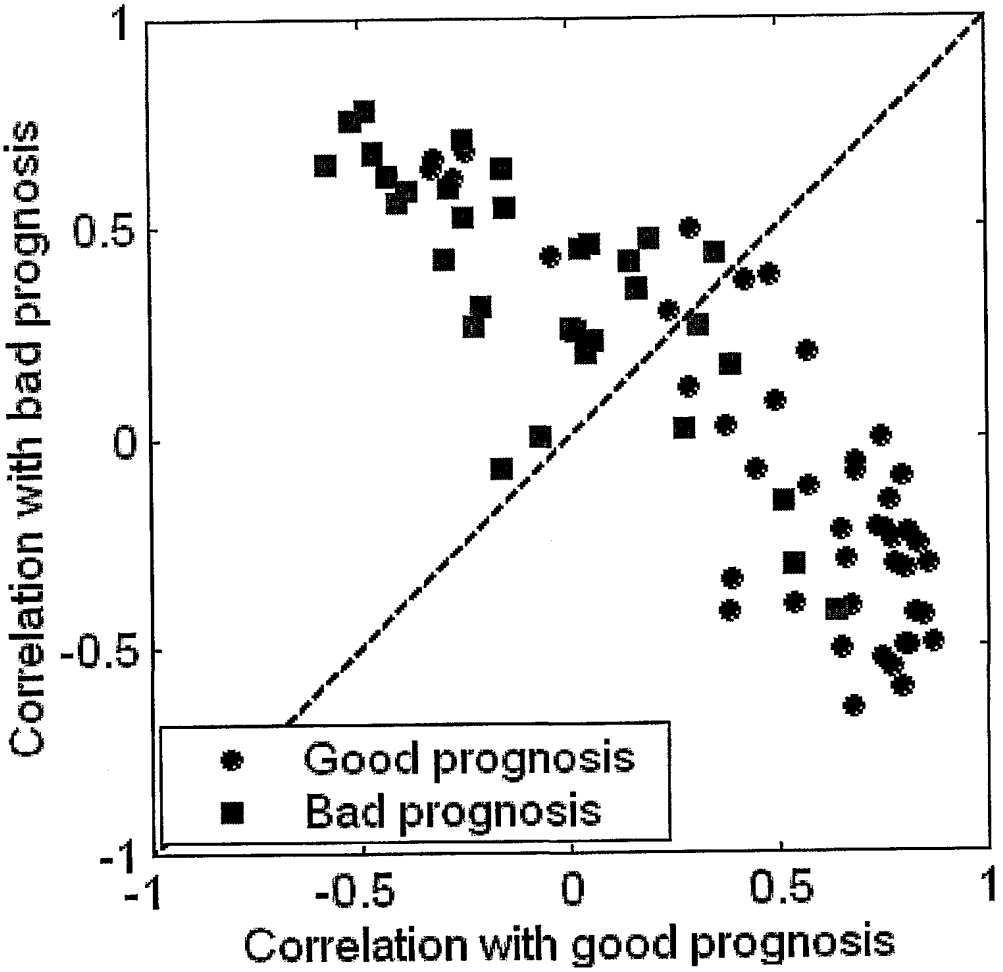
1
0.5
0
-0.5
-1
-1
-0.5
0
0.5
1
Correlation with bad prognosis
Correlation with good prognosis
Good prognosis
Bad prognosis

FIG. 15

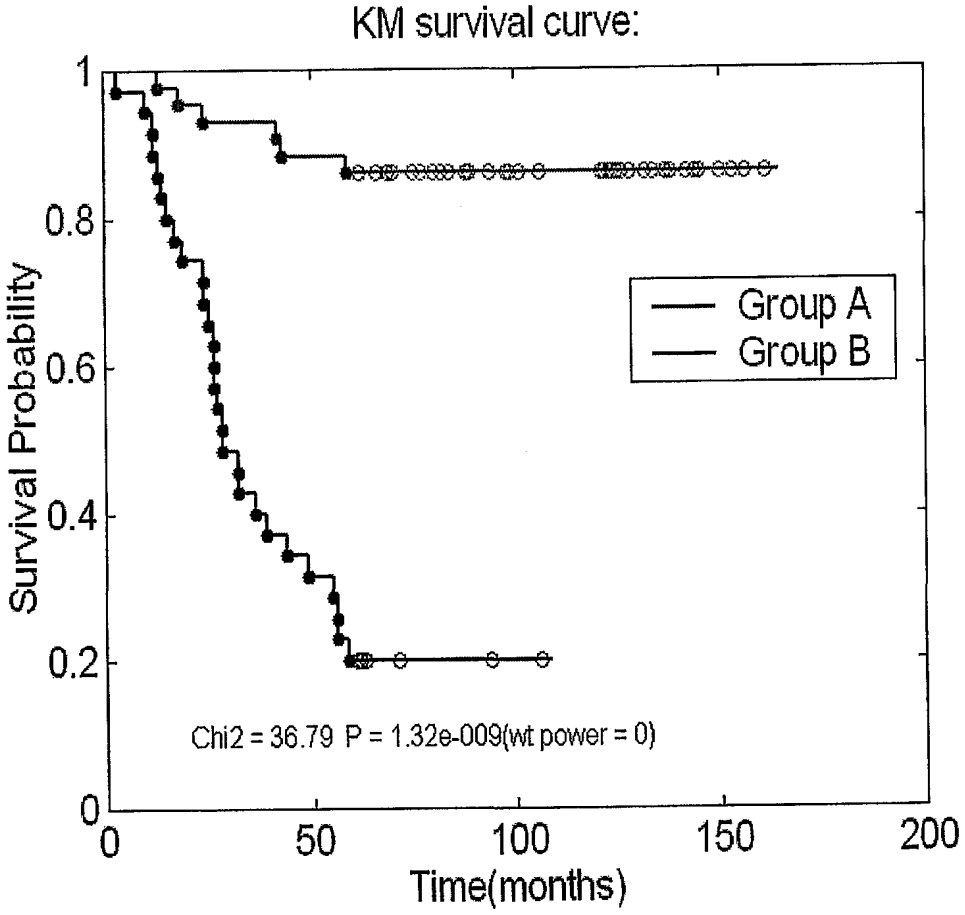
KM survival curve:
Survival Probability
Time(months)
Group A
Group B
Chi2 = 36.79 P = 1.32e-009(wt power = 0)
0
0.2
0.4
0.6
0.8
1
0
50
100
150
200

FIG. 16

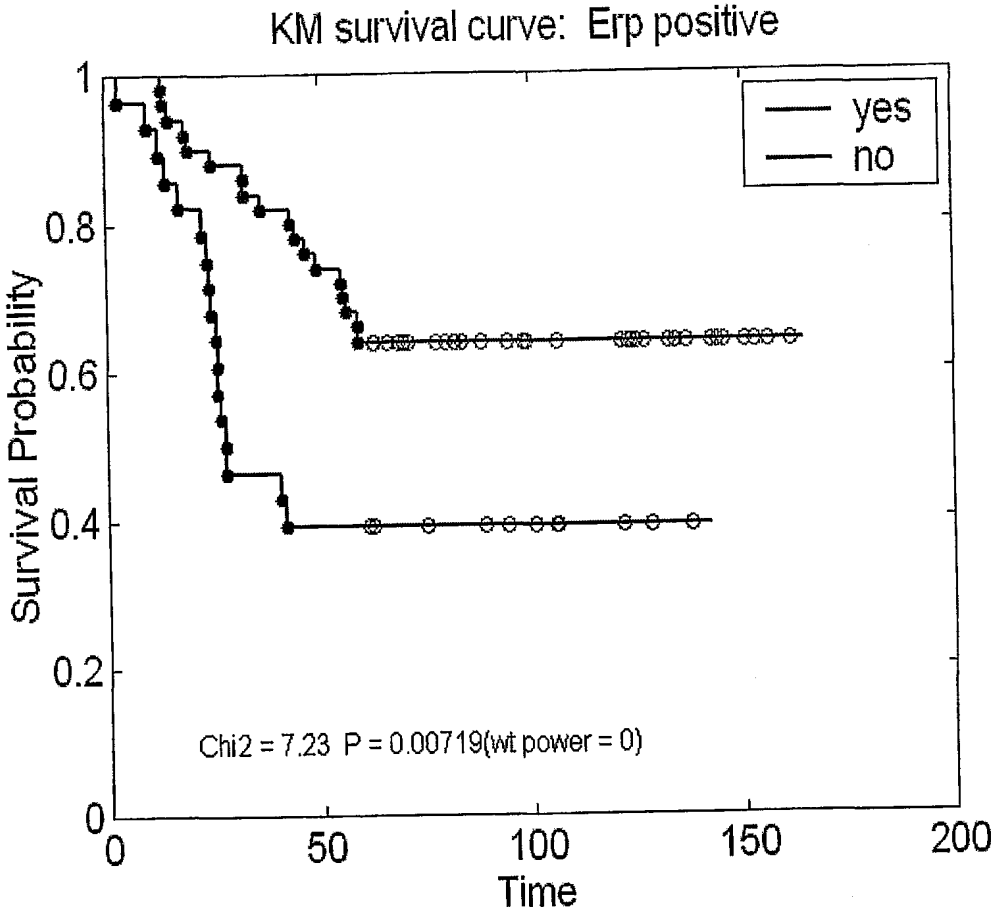
KM survival curve: Erp positive
yes
no
Survival Probability
1
0.8
0.6
0.4
0.2
0
Chi2 = 7.23 P = 0.00719(wt power = 0)
0
50
100
150
200
Time

FIG. 17

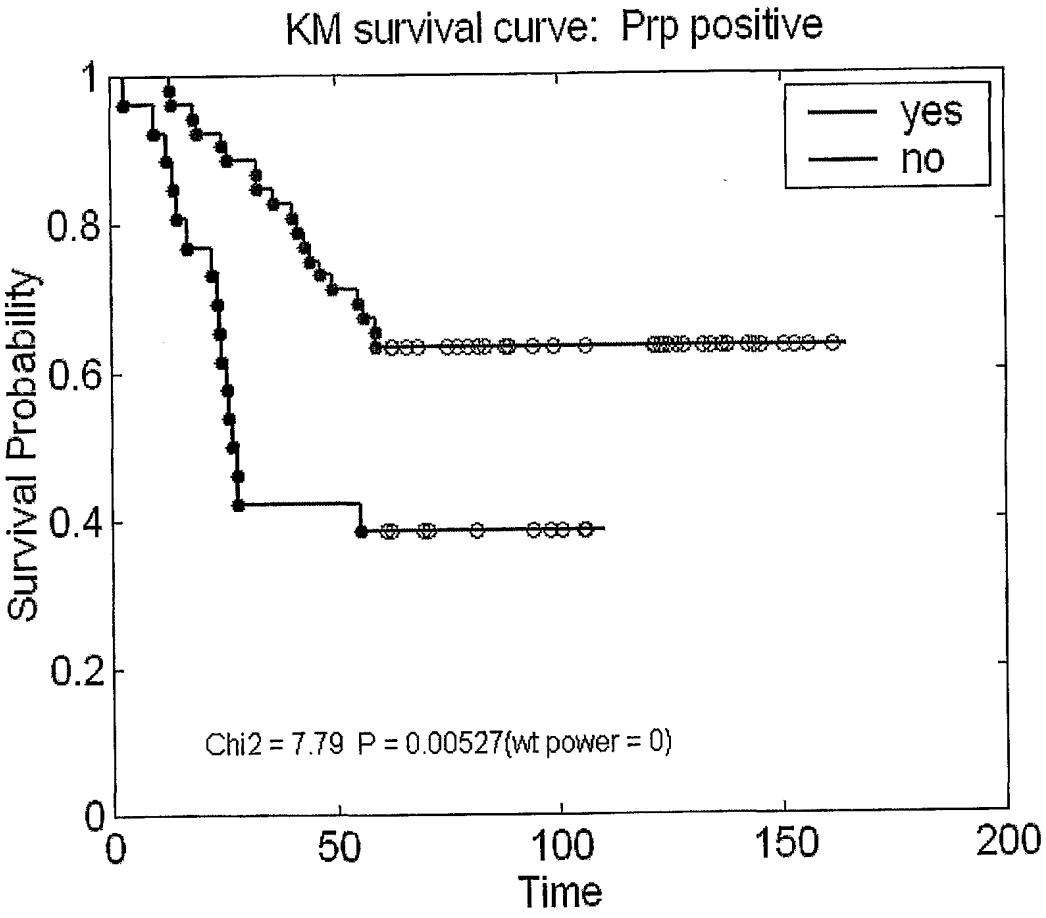
KM survival curve: Prp positive
yes
no
Survival Probability
1
0.8
0.6
0.4
0.2
0
0
50
100
150
200
Time
Chi2 = 7.79 P = 0.00527(wt power = 0)

FIG. 18

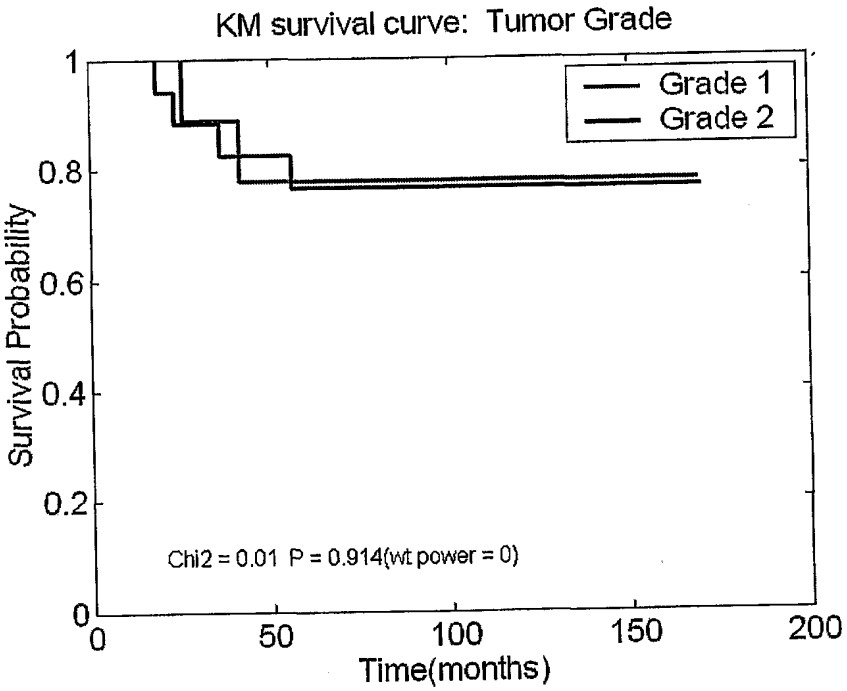
KM survival curve: Tumor Grade
Grade 1
Grade 2
Survival Probability
1
0.8
0.6
0.4
0.2
0
Chi2 = 0.01 P = 0.914(wt power = 0)
0
50
100
150
200
Time(months)

FIG. 19A

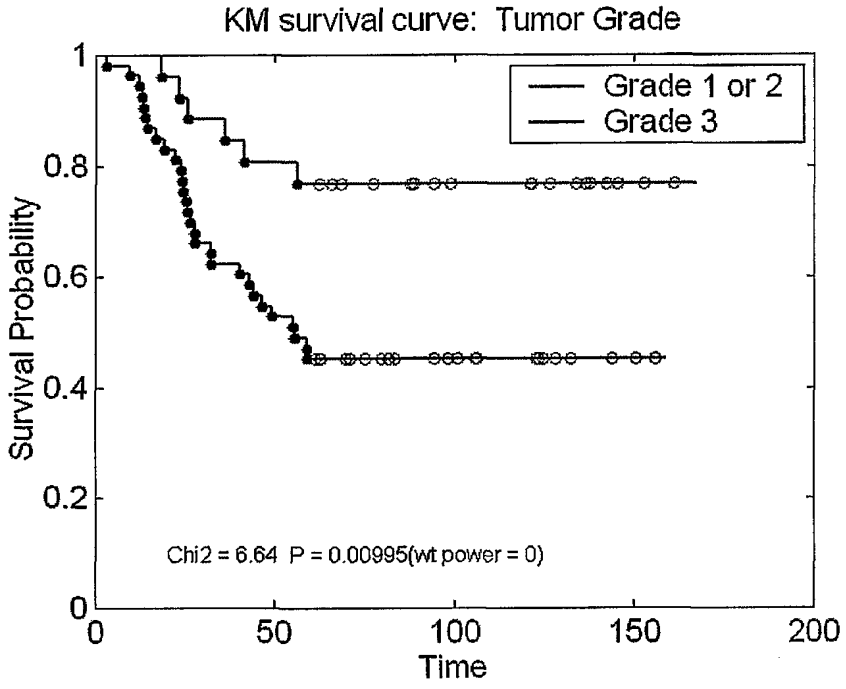
KM survival curve: Tumor Grade
Grade 1 or 2
Grade 3
Survival Probability
1
0.8
0.6
0.4
0.2
0
Chi2 = 6.64 P = 0.00995(wt power = 0)
0
50
100
150
200
Time

FIG. 19B

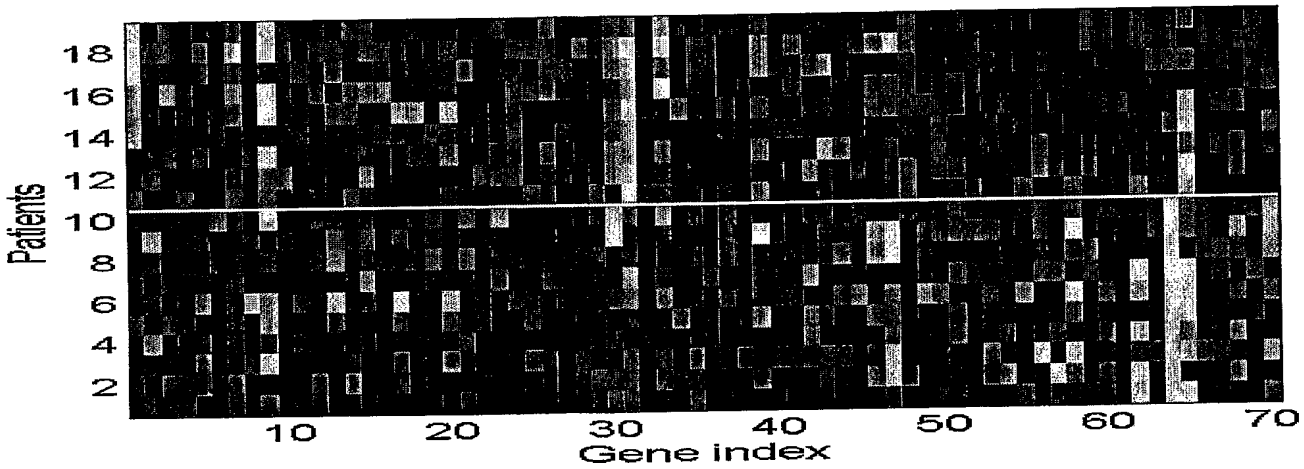

FIG. 20A

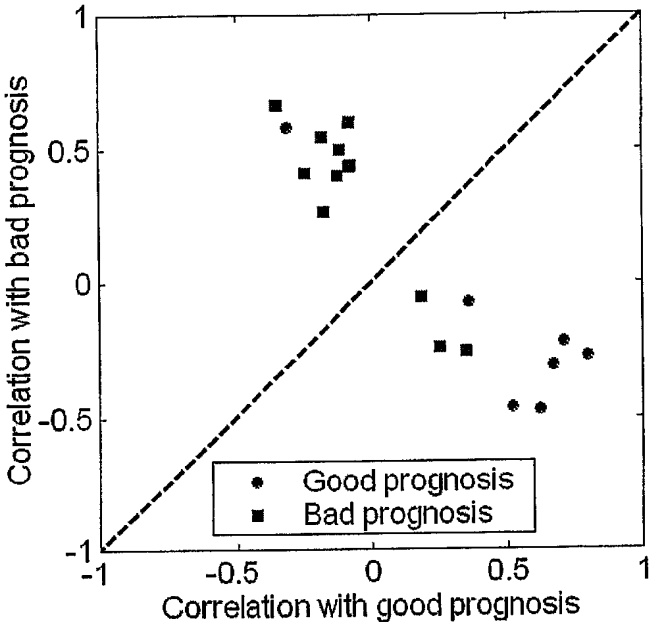
Correlation with bad prognosis
Correlation with good prognosis
1
0.5
0
-0.5
-1
-1
-0.5
0
0.5
1
Good prognosis
Bad prognosis

FIG. 20B

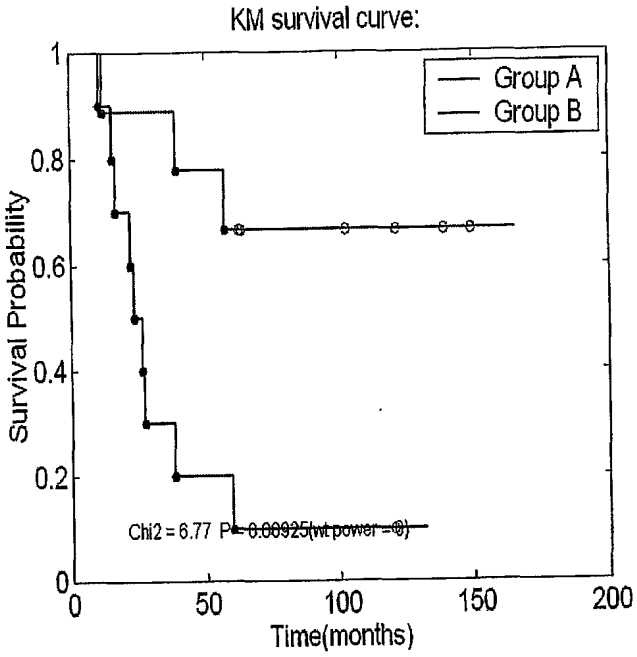
KM survival curve:
Group A
Group B
Survival Probability
1
0.8
0.6
0.4
0.2
0
0
50
100
150
200
Time(months)
Chi2 = 6.77 P= 0.00925(wt power =0)

FIG. 20C

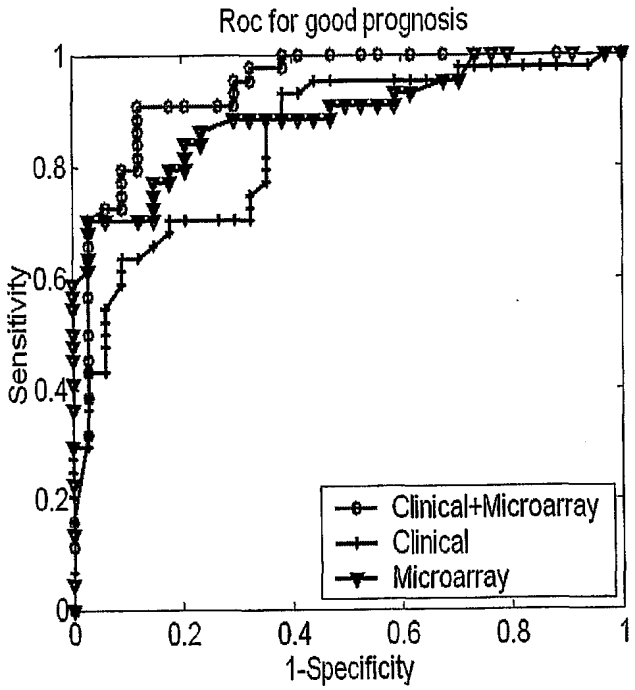
Roc for good prognosis
Sensitivity
1-Specificity
0
0.2
0.4
0.6
0.8
1
Clinical+Microarray
Clinical
Microarray

FIG. 21A

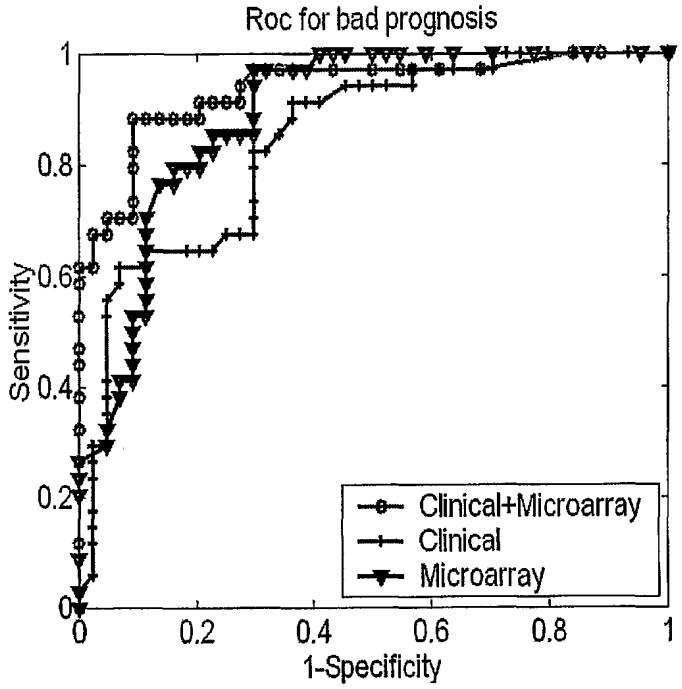
Roc for bad prognosis
Sensitivity
1-Specificity
0
0.2
0.4
0.6
0.8
1
Clinical+Microarray
Clinical
Microarray

FIG. 21B

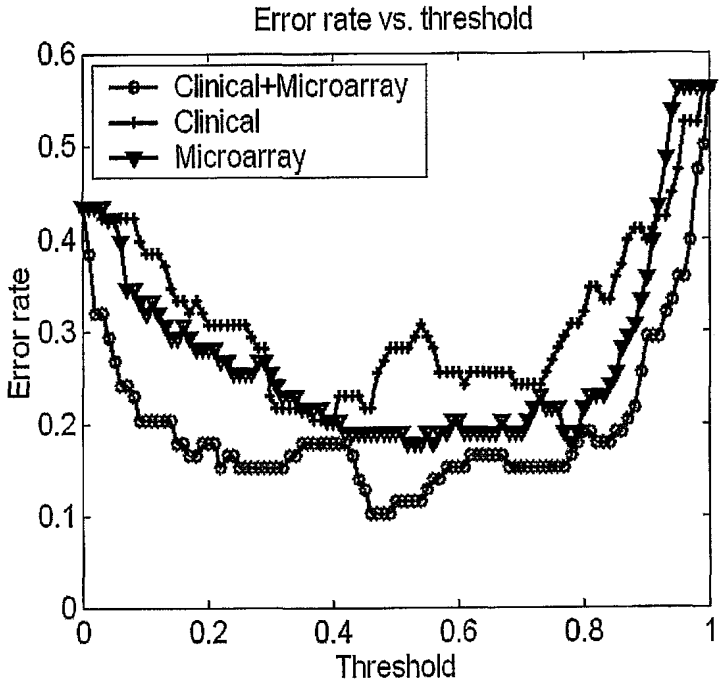
Error rate vs. threshold
Clinical+Microarray
Clinical
Microarray
Error rate
Threshold
0
0.1
0.2
0.3
0.4
0.5
0.6
0
0.2
0.4
0.6
0.8
1

FIG. 21C

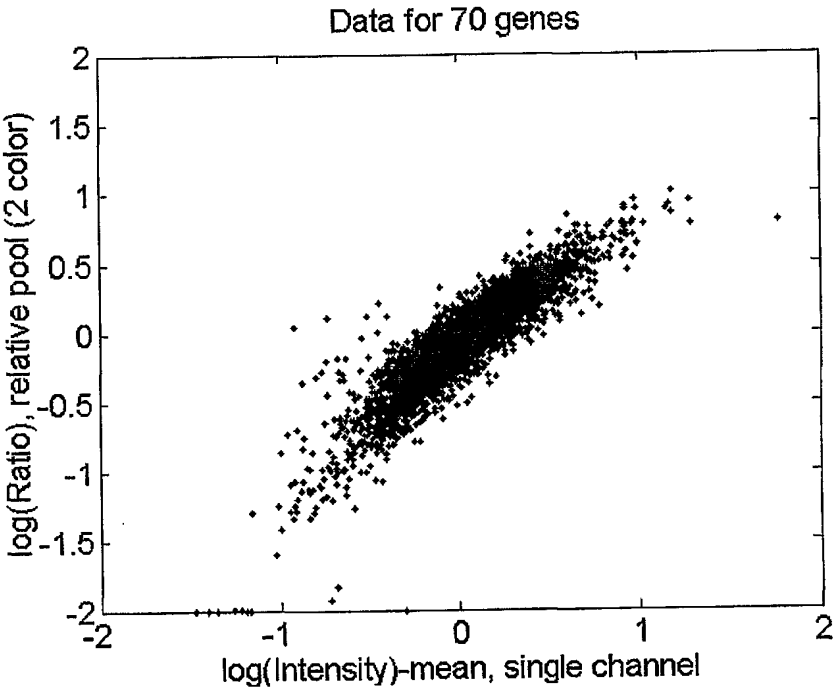
Data for 70 genes
log(Ratio), relative pool (2 color)
log(Intensity)-mean, single channel
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2
-1
0
1
2

FIG. 22

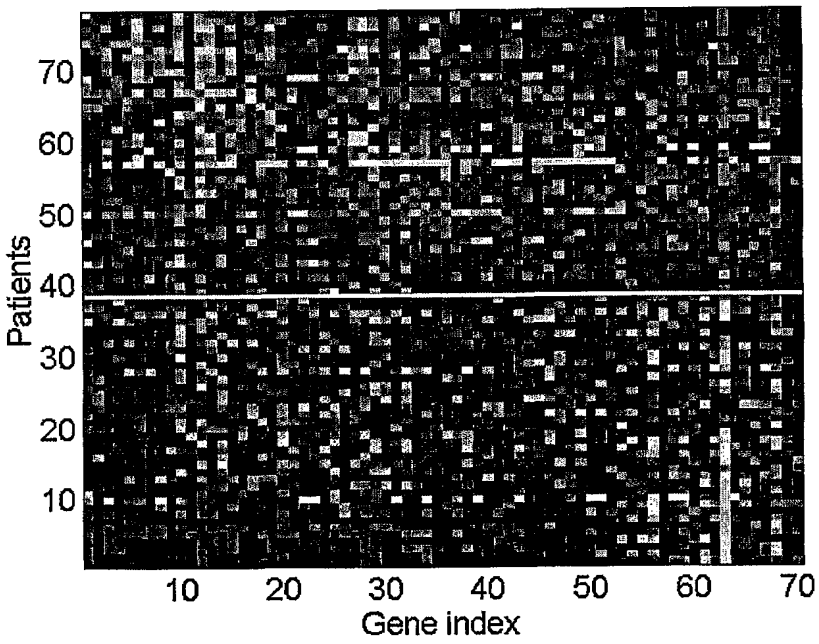

FIG. 23A

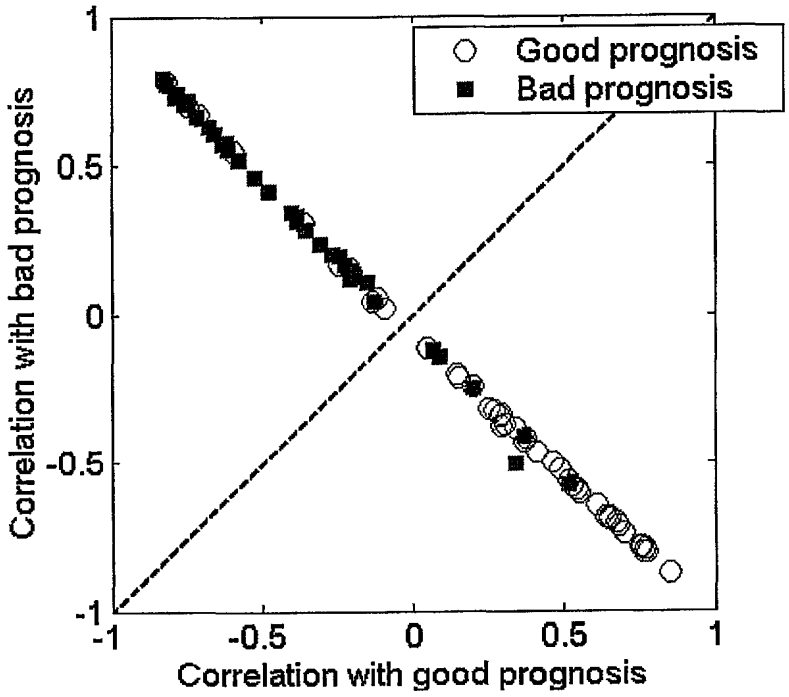
Good prognosis
Bad prognosis
Correlation with bad prognosis
Correlation with good prognosis
1
0.5
0
-0.5
-1
-1
-0.5
0
0.5
1

FIG. 23B

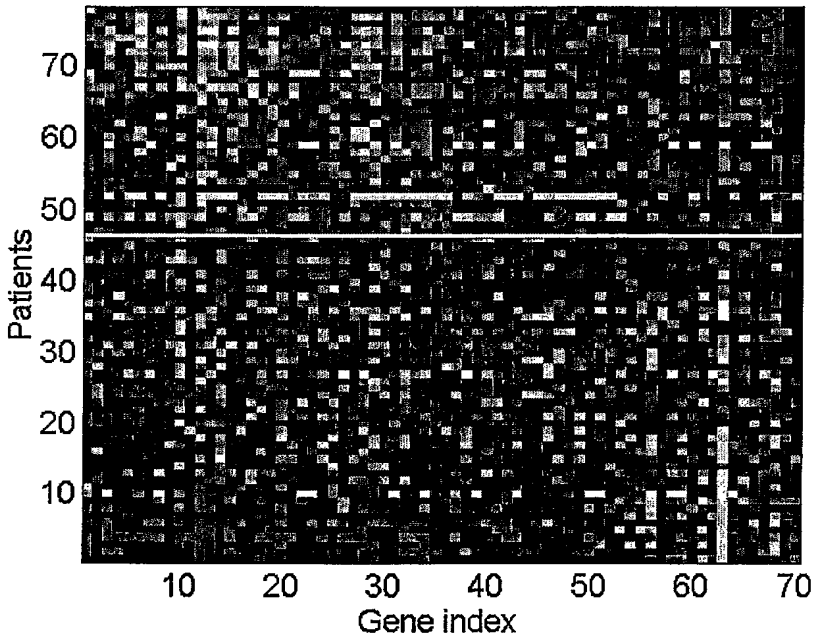

FIG. 24A

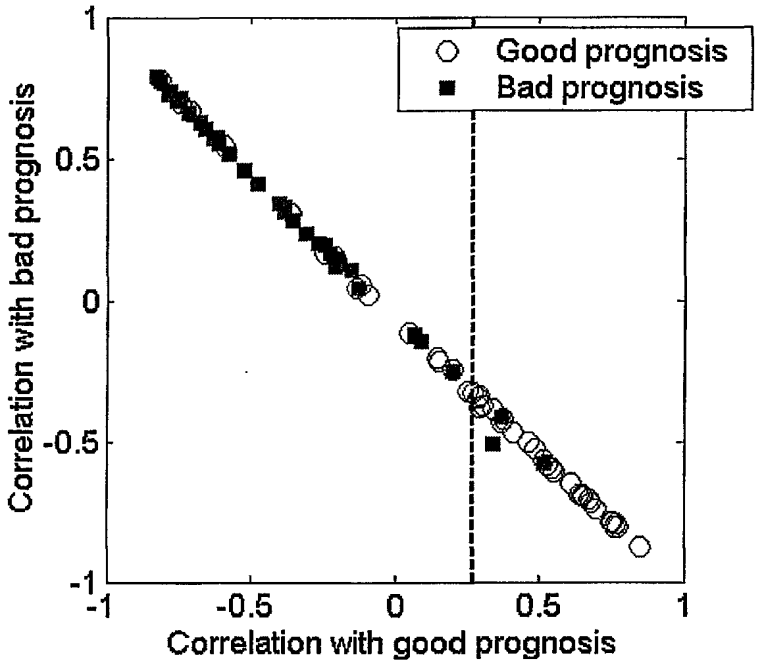
Good prognosis
Bad prognosis
Correlation with bad prognosis
Correlation with good prognosis
1
0.5
0
-0.5
-1
-1
-0.5
0
0.5
1

FIG. 24B

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(74) Agents: ANTLER, Adriane, M. et al.; Pennie & Edmonds LLP, 1155 Avenue of the Americas, New York, NY 10036 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report:
31 July 2003

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: DIAGNOSIS AND PROGNOSIS OF BREAST CANCER PATIENTS

(57) Abstract: The present invention relates to genetic markers whose expression is correlated with breast cancer. Specifically, the invention provides sets of markers whose expression patterns can be used to differentiate clinical conditions associated with breast cancer, such as the presence or absence of the estrogen receptor *ESR1*, and *BRCA1* and sporadic tumors, and to provide information on the likelihood of tumor distant metastases within five years of initial diagnosis. The invention relates to methods of using these markers to distinguish these conditions. The invention also relates to kits containing ready-to-use microarrays and computer software for data analysis using the statistical methods disclosed herein.

WO 02/103320 A3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/18947

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : G01N 33/48
US CL : 702/19
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 702/19

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EAST, STN

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X.P	SORLIE et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implicaitons. PNAS. 11 September 2001, Vol. 98, pages 10869-	11-18, 29,-30
A	LAIRD et al. Deficiency of conexin 43 gap junctions is an independent maker for breast tumors. Cancer Research. 15 August 1999, Vol. 59, pages 4104-4110.	1-60
A,P	US 6,342,491 B1 (DEY et al) 29 January 2002 (29.01.2002), columns 1-6.	1-10, 25, 27-28, 33-35
A,P	US 6,358,682 B1 (JAFFEE et al) 19 March 2002 (19.03.2002), columns 3-18.	1-60
A	US 6,107,034 A (WIEGEL, R.J.) 22 August 2000 (22.08.2000), columns 2-16.	41-48
A,P	US 6,342,483 B1 (HOLT et al) 29 January 2002 (29.01.2002), columns 12-32.	11-18, 29,-30
A,P	US 6,455,300 B1 (HTUN et al) 24 September 2002 (24.09.2002), columns 4-40.	1-60

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
07 February 2003 (07.02.2003)	13 MAY 2003
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Authorized officer Shubo "Joe" Zhou Telephone No. (703)-308-0196

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/18947

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I (claims 1-10, 25, 27-28, 33-35), drawn to methods of classifying a cell sample as ER(+) or ER(-).

Group II (claims 11-18, 29-30), drawn to methods of classifying a cell sample as BRACA-1 related or sporadic.

Group III (claims 19-24, 26, 31-32), drawn to methods of classifying an individual as having a good or poor prognosis.

Group IV (claims 36-39), drawn to methods of determining a set of marker genes whose expresion is associated with a particular phenotype.

Group V (claim 40), drawn to a method of assigning a person to one of a plurality of catergories in a clinical trial.

Group VI (claims 41-48), drawn to methods of classifying a cell or organism as having one of at least two different phenotypes.

Group VII (claims 49-60), drawn to microarrays comprising various protein markers.

The inventions listed as Groups I-VII do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reason:

Groups I-VII are unrelated to to each other because they have distinct special technical features. The special technical features for the groups are:

For group I, it is application of genes listed on Table 1. For group II, it is application of genes listed in Tables 3-4. For group III, it is application of genes listed in Table 5. For group IV, it is a combination of method steps involving an equation. For gorup V, it is application of genes listed in Table 6. For group VI, it involves comparison of expressions of a plurality of genes. For group VII, it is an microarray comprising various markers from Tables 1-6.

Additionally the claimed methods of groups I-VI comprise distinct method steps and produce distinct results, which are not coextensive and which do not share the same technical feature.

Thus, in summary, the inventions listed as Groups I-VII are not so lined under PCT Rule 13.1.